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United States Patent and Trademark Office

September 28, 2022

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APPLICATION NUMBER: ***11/722,018***
FILING DATE: ***June 18, 2007***
PATENT NUMBER: ***7713947***
ISSUE DATE: ***May 11, 2010***

Certified by

Kathi

Performing the Functions and Duties of the
Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

June 18, 2007

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Giampiero de Luca
Docket No.	:	SER-125
For	:	Cladribine Regimen for Treating Multiple Sclerosis

Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir:

It is respectfully requested that the above-identified patent application be amended as follows:

In the Specification

Please insert the following new paragraph after the Title of the invention on page 1, line 1:

Cross-Reference to Related Application

This application is the U.S. national stage application of International Patent Application No. PCT/EP2005/056954, filed December 20, 2005, which claims the benefit of U.S. Provisional Patent Application No. 60/638,669, filed December 22, 2004, the disclosures of which are hereby incorporated by reference in their entireties, including all figures, tables and amino acid or nucleic acid sequences.

After page 31: Please insert as new page 32 the attached Abstract of the Disclosure.

In the Claims

1-17 (canceled).

18 (new). A method of treating multiple sclerosis comprising the oral administration of a formulation comprising cladribine, wherein the formulation is to be orally administered following the sequential steps below:

(i) an induction period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the induction period is from 1.7 mg/kg to 3.5 mg/kg;

(ii) a cladribine-free period wherein no cladribine formulation is administered;

(iii) a maintenance period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the maintenance period is lower than the total dose of cladribine reached at the end of the induction period (i); and

(iv) a cladribine-free period wherein no cladribine formulation is administered.

19 (new). The method according to claim 18, wherein the induction period lasts up to 4 months, or up to 3 months, or up to 2 months.

20 (new). The method according to claim 19, wherein the induction period lasts up to 2 months.

21 (new). The method according to claim 18, wherein the induction period lasts up to 2 months.

22 (new). The method according to claim 18, wherein the induction period lasts up to 4 months.

23 (new). The method according to claim 19, wherein the induction period lasts up to 4 months.

24 (new). The method according to claim 18, wherein the total dose of cladribine reached at the end of the induction period is 1.7 mg/kg.

25 (new). The method according to claim 18, wherein the total dose of cladribine reached at the end of the induction period is 3.5 mg/kg.

26 (new). The method according to claim 18, wherein the cladribine-free period lasts up to 10 months, or up to 9 months, or up to 8 months.

27 (new). The method according to claim 18, wherein the cladribine-free (iv) period lasts up to 10 months.

28 (new). The method according to claim 18, wherein the maintenance period lasts up to 4 months, or up to 3 months or up to 2 months.

29 (new). The method according to claim 18, wherein the total dose of cladribine reached at the end of the maintenance period is 1.7 mg/kg.

30 (new). The method according to claim 18, wherein the formulation is to be orally administered following the sequential steps below:

(i) an induction period wherein said cladribine formulation is orally administered and wherein the total dose of cladribine reached at the end of the induction period is from 1.7 mg/kg to 3.5 mg/kg;

(ii) a cladribine-free period wherein no cladribine formulation is administered;

(iii) a maintenance period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the maintenance period is

lower than the total dose of cladribine reached at the end of the induction period (i); and

(iv) a cladribine-free period wherein no cladribine formulation is administered;

wherein the induction period lasts up to 4 months, or up to 3 months or up to 2 months; the cladribine-free period (ii) lasts up to 10 months, or up to 8 months or up to 10 months; the maintenance period (iii) lasts up to 2 months; the cladribine-free period (iv) lasts up to 10 months; the total dose of cladribine reached at the end of the maintenance period is 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

31 (new). The method according to claim 30, wherein the total dose of cladribine reached at the end of the induction period is 3.5 mg/kg and the total dose of cladribine reached at the end of the maintenance period is 1.7 mg/kg.

32 (new). The method according to claim 30, wherein the formulation is to be orally administered at a daily dose of 3 to 30 mg cladribine.

33 (new). The method according to claim 32, wherein the pharmaceutical formulation is to be orally administered at a daily dose of 10 mg cladribine.

34 (new). The method according to claim 18, wherein the pharmaceutical formulation is orally administered 1 to 7 days per month during the induction period.

35 (new). The method according to claim 18, wherein the steps (iii) to (iv) are repeated at least one or two times.

36 (new). The method according to claim 18, wherein said cladribine formulation is to be administered in combination with interferon-beta.

37 (new). The method according to claim 30, wherein said cladribine formulation is to be administered in combination with interferon-beta.

Remarks

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16, 1.17, and 1.492 as required by this paper to Deposit Account No. 19-0065.

Respectfully submitted,

/FRANKCEISENSCHENK/

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FCE/sl
Attachment: Abstract of the Disclosure

Abstract of the Disclosure

The present invention is related to the use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis, wherein the preparation is to be orally administered and wherein re-treatments are possible.

Application Information

Application Type::	Regular (National Stage)
Subject Matter::	Utility
Suggested Classification::	None
Suggested Group Art Unit::	None
CD-ROM or CD-R?::	None
Number of CD disks::	None
Number of copies of CDs::	None
Sequence submission?::	No
Computer Readable Form?::	No
Number of Copies of CRF::	None
Title::	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
Attorney Docket Number::	SER-125
Request for Early Publication::	No
Request for Non-Publication::	No
Suggested Drawing Figure::	None
Total Drawing Sheets::	None
Small Entity?::	No
Petition included?::	No
Petition Type::	N/A
Secrecy Order in Parent Appl.?::	No

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	Italy
Status::	Unknown
Inventor One Given Name::	Giampiero
Family Name::	DE LUCA
City of Residence::	Conches/Geneva
Country of Residence::	Switzerland
Street of Mailing Address::	Chemin des Conches 15B
City of Mailing Address::	Conches/Geneva
Country of Mailing Address::	Switzerland
Postal or Zip Code of Mailing Address::	CH-1231

Representative Information

Representative Customer Number::	000023557

Correspondence Information

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Telephone Number Two::	
Fax Number::	(352) 372-5800
Electronic Mail Address::	fce@slspatents.com

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This application is a	National Stage of	PCT/EP2005/056954	December 20, 2005
PCT/EP2005/056954	An application claiming the benefit under 35 USC 119(e) of	60/638,669	December 22, 2004

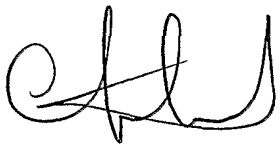
Foreign Priority Information

Country::	Application Number::	Filing Date::	Priority Claimed::
EP	04106909.7	December 22, 2004	Yes

Assignee Information

Assignee Name::	Laboratoires Serono S.A.
Street of Mailing Address::	Zone Industrielle de l'Ouriettaz
City of Mailing Address::	Aubonne
Country of Mailing Address::	Switzerland
Postal or Zip Code of Mailing Address::	CH-1170

VIII-4-1	**Declaration: Inventorship (only for the purposes of the designation of the United States of America)** Declaration of Inventorship (Rules 4.17(iv) and 51bis.1(a)(iv)) for the purposes of the designation of the United States of America:	I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought. This declaration is directed to international application PCT/EP2005/056954 (if furnishing declaration pursuant to Rule 26ter). I hereby declare that my residence, mailing address, and citizenship are as stated next to my name. I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.10, any claim to foreign priority, and I have identified below, under the heading "Prior Applications", by application number, country or Member of the World Trade Organization, day, month, and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.
VIII-4-1-1	Prior applications:	60/638,669, US, 22 December 2004 (22.12.2004) ;04106909.7, EP, 22 December 2004 (22.12.2004)

		I hereby acknowledge the duty to disclose information that is known by me to be material to patentability as defined by 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the PCT international filing date of the continuation-in-part application. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.
VIII-4-1-1-1	Name (LAST, First)	DE LUCA, Giampiero
VIII-4-1-1-2	Residence: (city and either US State, if applicable, or country)	Conches, Switzerland
VIII-4-1-1-3	Mailing address:	Chemin de Conches 15B 1231 Conches Switzerland
VIII-4-1-1-4	Citizenship:	IT
VIII-4-1-1-5	Inventor's Signature: (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)	
VIII-4-1-1-6	Date: (of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)	

June 18, 2007

INFORMATION DISCLOSURE
STATEMENT
Patent Application
Docket No. SER-125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Giampiero de Luca
Filed	:	June 18, 2007
For	:	Cladribine Regimen for Treating Multiple Sclerosis

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR §§1.97 AND 1.98

Sir:

In accordance with 37 C.F.R. § 1.56, the references listed on the attached form PTO/SB/08 are being brought to the attention of the Examiner for consideration in connection with the examination of the above-identified patent application. A copy of each cited reference is enclosed.

It is respectfully requested that the references cited on the attached form PTO/SB/08 be considered in the examination of the subject application and that their consideration be made of record.

Applicant respectfully asserts that the substantive provisions of 37 C.F.R. §§ 1.97 and 1.98 are met by the foregoing statement.

Respectfully submitted,

/FRANKCEISENSCHENK/

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Gainesville, FL 32614-2950

FCE/jps
Attachments: Form PTO/SB/08; copies of references cited therein.

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PTO/SB/08A (08-03)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet	1	of	3

Complete if Known	
Application Number	
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Art Unit	
Examiner Name	
Attorney Docket Number	SER-125

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No. [1]	Document Number Number - Kind Code [2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	U1	US-			
	U2	US-			
	U3	US-			
	U4	US-			
	U5	US-			
	U6	US-			
	U7	US-			
	U8	US-			
	U9	US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No. [1]	Foreign Patent Document Country Code [3] - Number [4] - Kind Code [5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T [6]
	F1	WO 04/087101 A2	10/14/2004	Ivax Corporation	All	
	F2	EP 0 626 853 B1	04/26/200	The Scripps Research Institute	All	
	F3					
	F4					
	F5					
	F6					
	F7					

Examiner Signature		Date Considered	

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This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08B (08-03)
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U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449B/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 2 of 3

Complete if Known	
Application Number	
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Group Art Unit	
Examiner Name	
Attorney Docket Number	SER-125

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No. [1]	Include name of the author (in CAPITAL LETTERS), title of the article, (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	R1	BEUTLER, E. *et al.* "Marrow Suppression Produced by Repeated Doses of Cladribine", *Acta Haematol*, 1994, pp. 10-15, Vol. 91.	
	R2	BEUTLER, E. *et al.* "Treatment of Multiple Sclerosis and Other Autoimmune Diseases With Cladribine", *Seminars in Hematology*, January 1, 1996, pp. 45-52, Vol. 33, No. 1, Supplement 1.	
	R3	BEUTLER, E. *et al.* "The treatment of chronic progressive multiple sclerosis with cladribine", *Proc. Natl. Acad. Sci. USA*, February 1996, pp. 1716-1720, Vol. 93.	
	R4	ELLISON, G. *et al.* "Oral Cladribine for Multiple Sclerosis", *Neurology*, March 1997, P03.070, pp. A174-A175, Vol. 48, No. 3, XP008047069.	
	R5	GRIEB, P. *et al.* "Effect of Repeated Treatments with Cladribine (2-Chlorodeoxyadenosine) on Blood Counts in Multiple Sclerosis Patients", *Archivum Immunologiae et Therapiae Experimentalis*, 1995, pp. 323-327, Vol. 43, No. 5-6.	
	R6	KAZIMIERCZUK, Z. *et al.* "Synthesis of 2'-Deoxytubercidin, 2'-Deoxyadenosine, and Related 2'-Deoxynucleosides via a Novel Direct Stereospecific Sodium Salt Glycosylation Procedure", *J. Am. Chem. Soc.*, 1984, pp. 6379-6382, Vol. 106, No. 21.	
	R7	KURTZKE, J. "Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS)", *Neurology*, November 1983, pp. 1444-1452, Vol. 33.	
	R8	LANGTRY, H. *et al.* "Cladribine: A Review of its Use in Multiple Sclerosis", *Biodrugs*, May 1998, pp. 419-433, Vol. 9, No. 3.	
	R9	LASSMANN, H. *et al.* "Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy", *TRENDS in Molecular Medicine*, March 2001, pp. 115-121, Vol. 7, No. 3.	
	R10	LUBLIN, F. *et al.* "Defining the clinical course of multiple sclerosis: Results of an international survey", *Neurology*, April 1996, pp. 907-911, Vol. 46.	
	R11	LUCCHINETTI, C. *et al.* "Multiple sclerosis: recent developments in neuropathology, pathogenesis, magnetic resonance imaging studies and treatment", *Current Opinion in Neurology*, 2001, pp. 259-269, Vol. 14.	
	R12	MATTSON, D. "Update on the diagnosis of multiple sclerosis", *Expert Review of Neurotherapeutics*, May 2002, pp. 319-327, Vol. 2, No. 3.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08B (08-03)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 3 of 3

Complete if Known	
Application Number	
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Group Art Unit	
Examiner Name	
Attorney Docket Number	SER-125

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No. [1]	Include name of the author (in CAPITAL LETTERS), title of the article, (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	R13	MCDONALD, W. *et al.* "Recommended Diagnostic Criteria for Multiple Sclerosis: Guidlines from the International Panel on the Diagnosis of Multiple Sclerosis", *Annals of Neurology*, July 2001, pp. 121-127, Vol. 50, No. 1.	
	R14	MILLER, R. *et al.* "Therapeutic advances in ALS", *Neurology*, 1996, pp. S217, Vol. 47, Suppl. 4.	
	R15	NOSEWORTHY, J. *et al.* "Multiple Sclerosis", *The New England Journal of Medicine*, September 28, 2000, pp. 938-952, Vol. 343, No. 13.	
	R16	POSER, C. *et al.* "New Diagnostic Criteria for Multiple Sclerosis: Guidelines for Research Protocols", *Annals of Neurology*, March 1983, pp. 227-231, Vol. 13, No. 3.	
	R17	RICE, G. *et al.* "Cladribine and progressive MS: Clinical and MRI outcomes of a multicenter controlled trial", *Neurology*, March 2000, pp. 1145-1155, Vol. 54.	
	R18	ROMINE, J. *et al.* "A Double-Blind, Placebo-Controlled, Randomized Trial of Cladribine in Relapsing-Remitting Multiple Sclerosis", *Proceedings of the Association of American Physicians*, *January*/February 1999, pp. 35-44, Vol. 111, No. 1.	
	R19	SCHUMACHER, G. *et al.* "Problems of Experimental Trials of Therapy in Multiple Sclerosis: Report by the Panel on the Evaluation of Experimental Trials of Therapy in Multiple Sclerosis", *Annals New York Academy of Sciences*, March 31, 1965, pp. 552-568, Vol. 122.	
	R20	SELBY, R. *et al.* "Safety and Tolerability of Subcutaneous Cladribine Therapy in Progressive Multiple Sclerosis", *Can. J. Neurol. Sci.*, 1998, pp. 295-299, Vol. 25.	
	R21	SIPE, J. *et al.* "A neurologic rating scale (NRS) for use in multiple sclerosis", *Neurology*, October 1984, pp. 1368-1372, Vol. 34.	
	R22	STELMASIAK, Z. *et al.* "A pilot trial of cladribine (2-chlorodeoxyadenosine) in remitting-relapsing multiple sclerosis", *Med. Sci Monit.*, 1998, pp. 4-8, Vol. 4, No. 1.	
	R23		
	R24		

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

Original Paper

Acta Haematol 1994;91:10–15

Marrow Suppression Produced by Repeated Doses of Cladribine

Ernest Beutler
James A. Koziol
Robert McMillan
Jack C. Sipe
John S. Romine
Carlos J. Carrera

Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, Calif., USA

Key Words
Aplastic anemia
2-Chlorodeoxyadenosine
Stem cell
Thrombocytopenia
Toxicity

Abstract
2-Chlorodeoxyadenosine (cladribine, Leustatin®) is being used extensively in the treatment of hematologic malignancies, but relatively little is known regarding its toxicity to the normal marrow. Long-term serial hematologic observations have been made on 29 patients with multiple sclerosis undergoing experimental therapy with monthly courses of cladribine, each of which consisted of 0.087–0.1 mg/kg per day for 7 days. The characteristic hematologic responses of the patients consisted of acute transient monocytopenia, prolonged, profound lymphopenia especially of CD4-positive cells, and modest lowering of the granulocyte count and hemoglobin with development of long-lasting macrocytosis. Two patients developed severe aplastic anemia, requiring transfusion both of red cells and platelets. One of these had previously received extensive therapy with chlorambucil, while the other had received carbamazepine (Tegretol®) and was ingesting phenytoin (Dilantin®) at the time of cladribine therapy. Both patients recovered after several months of marrow suppression.

2-Chlorodeoxyadenosine (cladribine, Leustatin®) is a remarkably effective and, in most patients, relatively nontoxic deoxyadenosine analogue that has recently been licensed for the treatment of hairy cell leukemia. While patients with hairy cell leukemia generally respond to a single course of treatment with cladribine, other lymphoid disorders such as low-grade lymphoma and chronic lymphocytic leukemia are also responsive to this drug, but several courses of therapy, typically given a month apart, are generally required to achieve remission [1].

The existence of stem cell toxicity giving rise to thrombocytopenia, and to a lesser extent anemia and leukopenia, was recognized early in our investigations of this drug, and tended to occur in an idiosyncratic manner. Since most of the patients given cladribine suffered from hematologic malignancy with marrow involvement or had previously received extensive marrow-suppressive therapy, it has always been difficult to ascertain whether marrow hypoplasia, when it occurred, was due to the drug or to the basic disease and its prior treatment.

We have now had the opportunity to study the effect of the administration of cladribine to 29 hematologically normal patients being treated experimentally for chronic progressive multiple sclerosis in the course of a double-blind crossover study. Two patients developed severe, long-lasting but reversible marrow hypoplasia, while 6 other patients developed moderate anemia, thrombocytopenia, and neutropenia.

Received:
November 11, 1993
Accepted:
December 17, 1993

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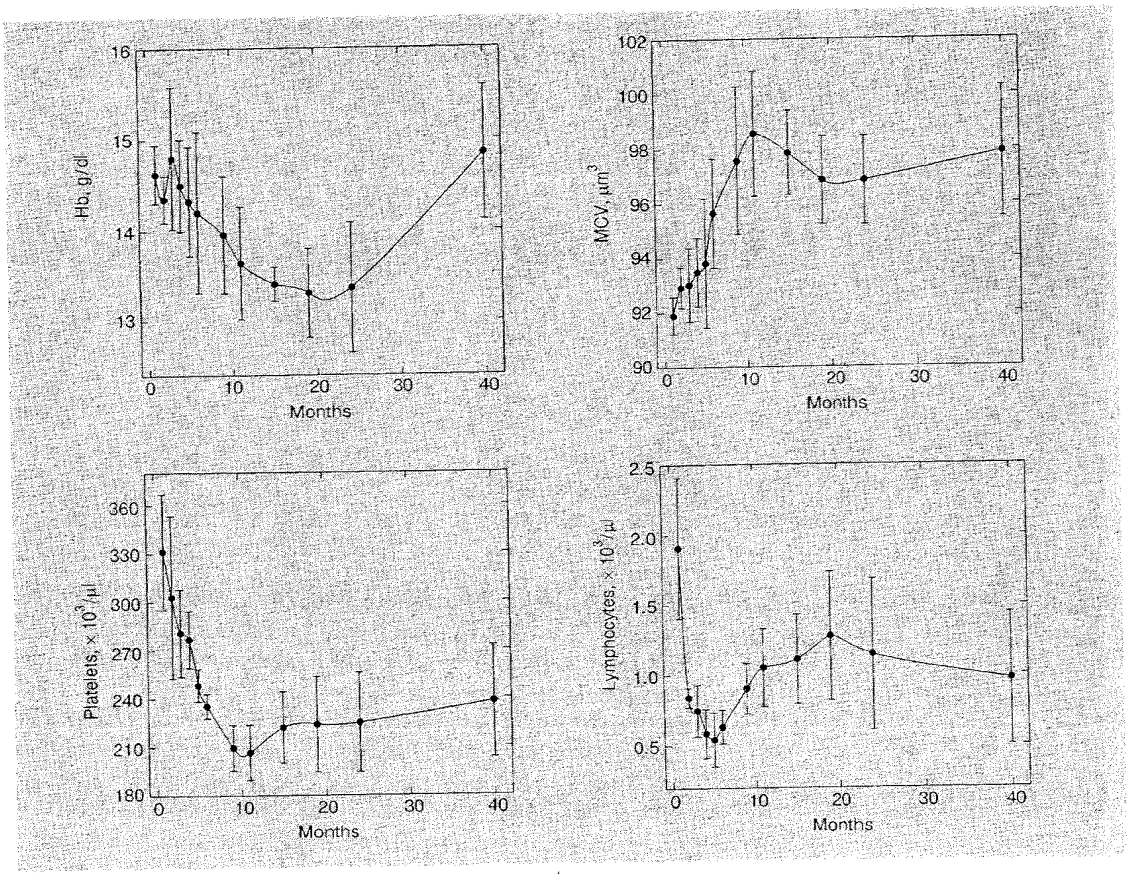


Fig. 1. The effect of the administration of six courses of cladribine, each consisting of 7 days of 0.087 mg/kg/day, on the hemoglobin level, MCV of the red cells, platelet count, and absolute lymphocyte count of patients 1–4. These patients were in the group in which there was 3-year follow-up. The bars represent one standard error.

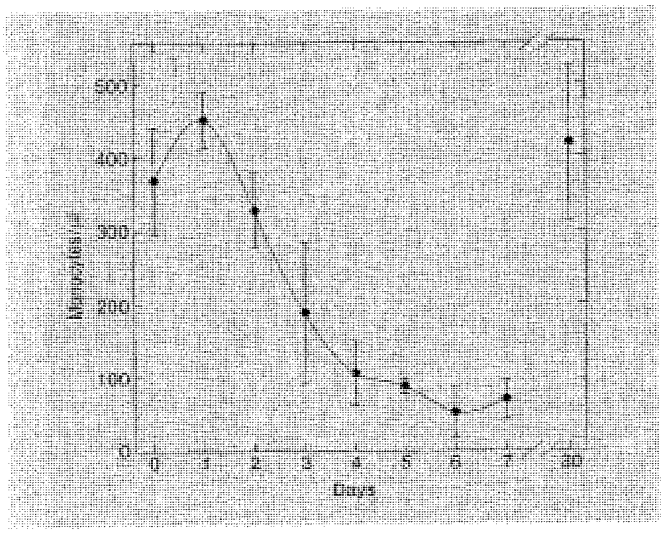
Treatment Protocol

All patients were hematologically normal adults with chronic progressive multiple sclerosis. Cladribine was administered by continuous infusion through a Portacath® (Bard Access Systems, MRI Port, Cranston, R.I., USA) for 7 days. Patients 1–4 received six cycles of drug, 1 month apart, each cycle consisting of 0.087 mg/kg/day for 7 days. Patients 5 and 6 received 3 cycles of 0.087 mg/kg/day for 7 days. Patient 7 was given 0.1 mg/kg/day in five 7-day cycles, 1 month apart, and patients 8–29 were placed on a protocol consisting of only 4 such cycles. Among these latter 22 patients, drug therapy was stopped in 1 case because of a fall in the platelet count after only two courses. A complete blood count and a chemistry panel were obtained on each patient before each infusion of drug. Age, sex, and disease severity-matched multiple sclerosis patients were matched to patients 7–29, and were given saline placebo. Hematologic values obtained on these patients served as control values relative to patients 7–29.

Methods and Results

The effect of cladribine on the blood counts of patients 1–4, who received six courses of drug, is shown in figures 1 and 2. Monocyte counts were measured only during the first cycle of drug administration, since in subsequent cycles daily blood counts were not obtained. Relatively striking macrocytosis, persisting in some cases for over 3 years after drug administration, was observed, and persistent lymphopenia was also documented. However, none of the patients became thrombocytopenic and very mild anemia was observed in only 1 patient.

The hematologic effect of cladribine administration to patients 7–29 and to matched controls is summarized in figure 3. Follow-up in these patients averaged 10.8 months.



Average hemoglobin and hematocrit fell during therapy, reaching a nadir at about 8 months (4 months after conclusion of treatment), a statistically significant decline relative to the controls ($p = 0.026$ from a distribution-free test for repeated measures) [2]. The lowest hemoglobin level observed was 3.2 g/dl in a transfused patient (10) described in further detail later.

MCV rose linearly for about 4 months following onset of therapy, and continued to increase at a more gradual rate after cessation of therapy. Differences between the treatment and the control group were statistically significant ($p = 0.042$) [2].

Fig. 2. The effect of the first cycle of cladribine therapy given to patients 1–4 on the blood monocyte counts. The bars represent one standard error.

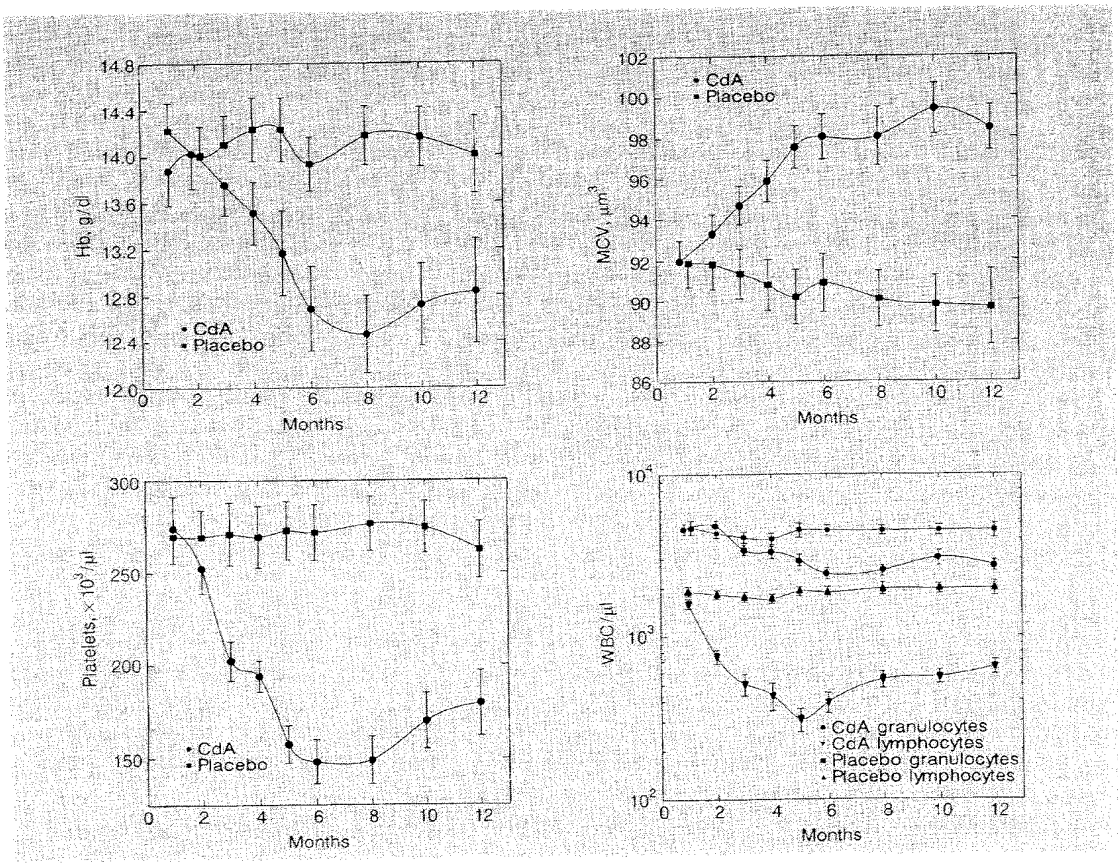


Fig. 3. The effect of cladribine (CdA) administration on serial blood hemoglobin level, MCV of the red cells, platelet count, total granulocyte count and total lymphocyte count of patients 7–29 and matched controls. The bars represent one standard error.

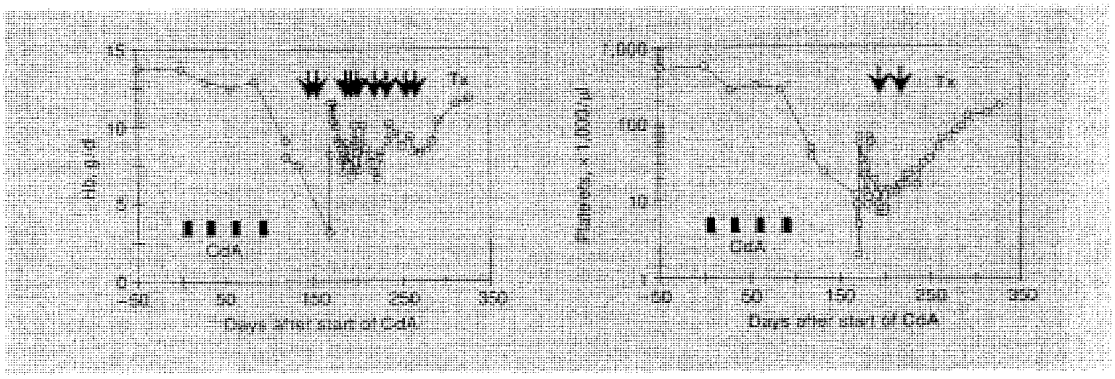


Fig. 4. The serial blood count of patient 10, who experienced the most profound marrow suppression in connection with cladribine (CdA) administration. This patient had also received Tegretol and was ingesting large doses of dilantin concurrently with and following cladribine therapy. Tx signifies a platelet or red cell transfusion. Each block represents a 7-day course of cladribine.

Granulocyte levels fell substantially following the second course of cladribine, and reached a nadir between 6 and 8 months. In contrast, lymphocyte levels plunged immediately after the first infusion of cladribine, reached a nadir at about 5 months and began to recover by about 6 months. With both parameters, differences between the treatment and the control group were statistically significant ($p = 0.001$ with granulocytes, $p < 0.00001$ with lymphocytes) [2]. The lowest granulocyte count was $1.03 \times 10^3/\mu l$ at 6 months. The lowest lymphocyte count was $0.01 \times 10^3/\mu l$, observed in patient 16 at month 5. Although lymphopenia was observed in all patients, infectious complications were not a serious problem. Two patients developed segmental herpes zoster. They were treated with oral acyclovir, and their lesions regressed rapidly without dissemination.

Platelet levels decreased sharply for about 6 months following onset of therapy, and tended to reach a nadir at about 8 months. Again, differences between the treatment and the control group were statistically significant ($p < 0.0001$). In 7 patients, platelet counts declined to below 100,000/µl, and in 4 of these patients minimum counts below 80,000 were observed, the lowest count being 2,000/µl in patient 10.

Two patients became severely anemic and thrombocytopenic, 1 after her third course of cladribine and the other after the fourth course. In each case there were factors that might have predisposed to marrow suppression, although the factors in the two patients were different. Patient 5 had been treated with a total of 950 mg of chlorambucil in the previous year. Patient 10, who had severe trigeminal neuralgia, had taken 1,200 mg/day of carbamazepine (Tegretol®) for 2 months, terminating 4 months before cladribine therapy was started. She took 550 mg/day of Dilantin from the 2nd month of therapy. Although Dilantin therapy was discontinued by her physicians when thrombocytopenia and anemia were first noted 5 months after therapy had started, the patient continued ingesting dilantin surreptitiously until she was admitted to the hospital with a hemoglobin of 3.2 g/dl and a platelet count of 12,000/µl a month later. In each case marrow biopsy revealed a hypocellular marrow containing about 90% fat on section and very few megakaryocytes. Patient 5 had unsuspected gastric angiodysplasia that caused repeated bleeding episodes when her platelet count had declined to only about 50,000/µl. The further fall in her platelet count to 10,000/µl may have been related to the bleeding. Patient 10, however, had no bleeding episodes, even though a platelet count as low as 2,000/µl was observed. She required red cell transfusions and prophylactic platelet transfusions were administered only on two occasions, once when her platelet count fell to 2,000/µl and a second time when she became febrile with a platelet count of 12,000/µl. Both patients received therapy with GM-CSF, but there was no notable effect of this treatment on either the leukocyte or the platelet count. The hematologic course of patient 10 is summarized in figure 4.

Mononuclear leukocytes from 6 patients receiving cladribine (patients 13, 14, 15, 21, 23, 24) and 6 control patients (controls 7, 13, 18, 19, 21, 26) were obtained prior to the monthly cladribine infusions and up to 1 year from the start of therapy. Cells were isolated from heparinized pe-

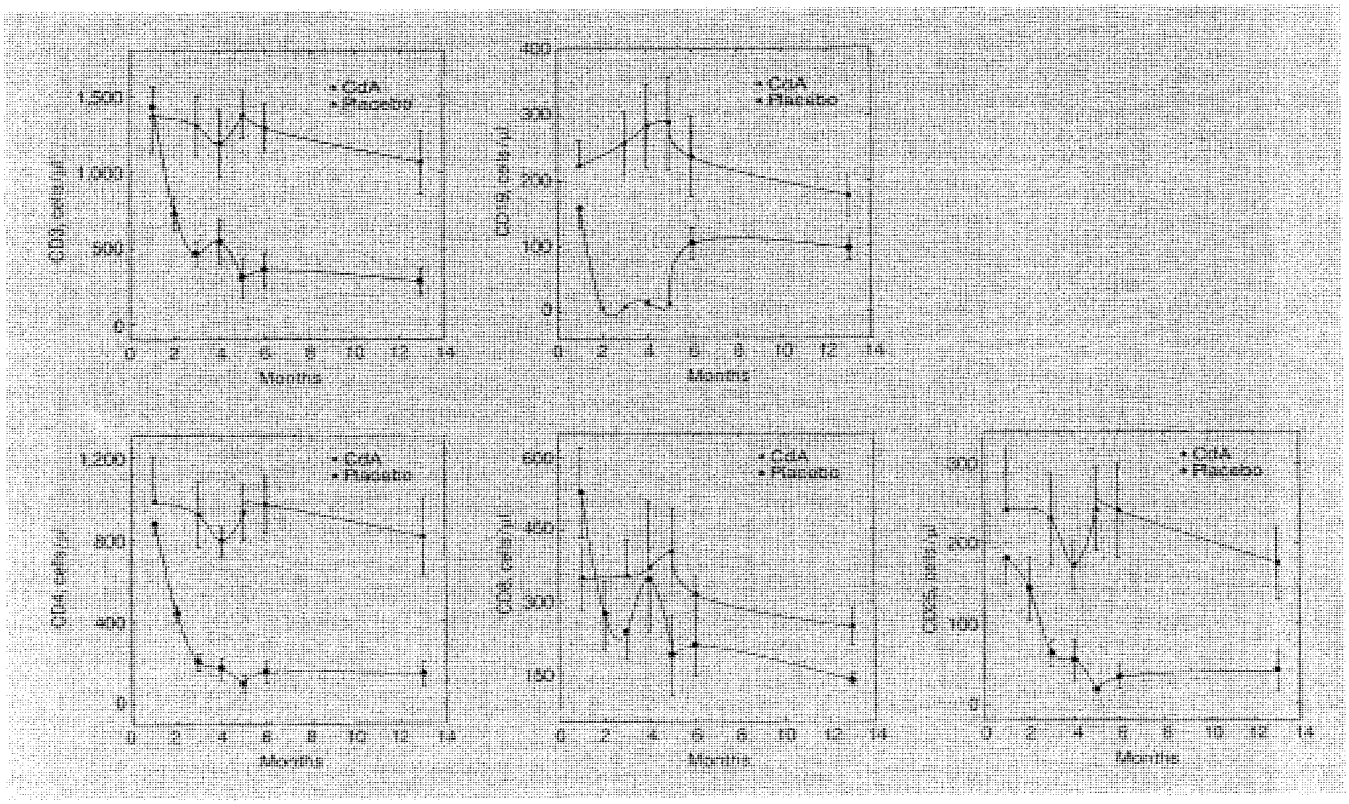


Fig. 5. Results of serial cytometric analysis of 6 patients receiving cladribine (CdA) and of 6 controls. The levels of cells positive for CD3, CD19, CD4, CD8, and CD25 are shown. The 13-month point in the 2-CdA group represents the only two patient samples that were available at the time of testing. The bars represent one standard error. Each patient receiving cladribine was given 0.1 mg/kg/day for 7 days in each of four monthly courses. Methods are given in the text.

ripheral blood by centrifugation over Histopaque (Sigma, St. Louis, Mo., USA) and were cryopreserved until analysis. Fluorochrome-conjugated monoclonal antibodies reactive for CD3, CD4, CD8, CD19, and CD25 were obtained from Becton Dickinson Immunocytometry Systems (San José, Calif., USA). For determination of surface phenotype, patient and control normal mononuclear cells were washed and suspended in phosphate-buffered saline containing NaN_3 0.01%. Cells were stained for 30 min at 4 °C with saturating amounts of the monoclonal antibodies against various surface antigens, or with isotype-specific control antibodies. Propidium iodide was added at 1 μg/ml to stain dead cells. After washing to remove unbound antibody, cells were analyzed on a FACScan® flow cytometer (Becton Dickinson and Co.) using Consort 30 software. Viable lymphocytes were distinguished by forward and orthogonal light scatter properties.

Lymphocyte subset analysis was undertaken on these 12 patients. It is apparent from figure 5 that the initial lymphocyte depletion caused by cladribine affects both T (CD3+) and B (CD19+) lymphocytes equally. Partial B lymphocyte recovery is evident 2 months after the last infusion, whereas T lymphocytes remain markedly depressed for at least 1 year after the start of therapy. Both parameters are significantly lower among the cladribine group compared with the control group ($p = 0.027$ and 0.029 with CD3+ and CD19+, respectively). Helper-inducer T cells (CD4+) are significantly depleted during cladribine therapy, and remain depressed at 12 months ($p = 0.013$). Cytotoxic-suppressor T cells (CD8+) follow the same pattern; however, differences between the treatment and control group here are not statistically significant ($p = 0.276$). Importantly, the subset of activated T lymphocytes expressing the receptor for interleukin-2 (CD25+) is also depleted to subnormal levels by cladribine infusions relative to control values ($p = 0.036$).

Discussion

Treatment of patients without hematologic disease with cladribine demonstrated that this drug has the capacity to produce a mild depression of the platelet count and granulocyte count in most patients, transient monocytopenia, long-lasting and sometimes profound lymphopenia, and long-lasting macrocytosis. In contrast to the usually transient changes in myeloid cells, the suppression of lymphocyte counts and particularly of CD4+ cells was profound and sustained for over a year. Notably, infectious episodes in these patients were limited to mild episodes of herpes zoster; no other opportunistic infections were encountered.

While we have previously observed marrow suppression in patients receiving cladribine, pancytopenia was an unusual occurrence and always occurred in the context of a disease that could have itself caused marrow dysplasia. Pancytopenia has not been a notable finding of others administering this drug in multiple courses to a variety of patients with hematologic diseases [3–7]. However, we recently found that 1 of 7 solid tumor patients given two courses of cladribine, 0.1 mg/kg/day for 7 days, developed severe thrombocytopenia [8]. This patient with far advanced malignant melanoma had been extensively pretreated with interleukin-2, interferon, and with lymphokine-activated killer cells.

Seven percent of the multiple sclerosis patients in the present study developed severe marrow suppression. In view of the mild hematologic changes that we observed in the first 4 patients who each received six courses of cladribine, we were surprised at the occurrence of severe marrow suppression in patients 5 and 10, who received only three and four courses of drug. However, the drug given to the patients who developed marrow suppression was obtained from a commercial source (Ortho Biotech), not synthesized in our department as had been done in some of the earlier studies. As a result of a change in standardization, the dose was about 12% higher than in previous clinical trials in which the actual dose administered, while reported to have been 0.1 mg/kg, is now estimated to have actually been only 0.087 mg/kg [1]. It seems doubtful that this slight difference in dosage would have such an effect, particularly since the patients who received only 0.087 mg/kg/day were given six courses of drug for a total dose of 37.8 mg/kg, while 1 patient developing severe suppression received only 28 and the other 21 mg/kg. Possibly patients with multiple sclerosis might be more sensitive to the marrow-suppressive effect of cladribine because most of them were ingesting multiple drugs for the treatment of manifestations of their primary disorder. Indeed, patient 5 had been treated with a very large total dose of chlorambucil and both Tegretol and Dilantin, ingested by patient 10, are known to produce marrow aplasia.

The observations we now report indicate that the potential of cladribine to produce marrow suppression had been somewhat underestimated. Particularly notable is the late onset of this complication and the severe degree of marrow suppression that can be present even 4–8 months after the drug has no longer been given. The fact that a patient has a normal blood count when a course is given in no way negates the possibility of this complication occurring.

Acknowledgement

The authors gratefully acknowledge the assistance of the staff of the General Clinical Research Center and of Ms. Kendis Cox, who carried out the lymphocyte flow cytometric analysis. This work was supported by FDA grant FD-R-000280 and NIH grant numbers NS30218 and RR00833, and the Sam Stein and Rose Stein Charitable Trust Fund.

References

1 Beutler E: Cladribine (2-chlorodeoxyadenosine). Lancet 1992;340:952–956.

2 Koziol JA, Maxwell DA, Fukushima M, Colmerauer MEM, Pilch YH: A distribution-free test for tumor-growth curve analyses with application to an animal tumor immunotherapy experiment. Biometrics 1981;37:383–390.

3 Dimopoulos MA, Kantarjian H, Estey E, O'Brien S, Delasalle K, Keating MJ, Freireich EJ, Alexanian R: Treatment of Waldenström macroglobulinemia with 2- chlorodeoxyadenosine. Ann Intern Med 1993;118:195–198.

4 Hickish T, Serafinowski P, Cunningham D, Oza A, Dorland E, Judson I, Millar BC, Lister TA, Roldan A: 2′-Chlorodeoxyadenosine: Evaluation of a novel predominantly lymphocyte selective agent in lymphoid malignancies. Br J Cancer 1993;67:139–143.

5 Juliusson G, Elmhorn-Rosenberg A, Liliemark J: Response to 2-chlorodeoxyadenosine in patients with B-cell chronic lymphocytic leukemia resistant to fludarabine. N Engl J Med 1992; 327:1056–1061.

6 Juliusson G, Lilienmark J: Complete remission of B-cell lymphocytic leukemia after oral cladribine. Lancet 1993;341:54.

7 Santana VM, Mirro J Jr, Kearns C, Schell MJ, Crom W, Blakley RL: 2-Chlorodeoxyadenosine produces a high rate of complete hematologic remission in relapsed acute myeloid leukemia. J Clin Oncol 1992;10:364–370.

8 Saven A, Kawasaki H, Carrera CJ, Waltz T, Copeland B, Zyroff J, Kosty M, Carson DA, Beutler E, Piro LD: 2-Chlorodeoxyadenosine dose escalation in nonhematologic malignancies. J Clin Oncol 1993;11:671–678.

Treatment of Multiple Sclerosis and Other Autoimmune Diseases With Cladribine

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CLADRIBINE (2-chlorodeoxyadenosine) was developed by Carson et al[4-8] as a nucleoside analog with high specificity for lymphocytes, with the hope that it might prove useful in treating patients with lymphoid neoplasia and patients with autoimmune disease; the specificity of this compound for lymphocytes is a consequence of the high deoxycytidine kinase and low 5'-nucleotidase activity of these cells. Cladribine has been studied extensively in neoplastic disease and found to be very effective in the treatment of low-grade lymphoid disorders, but relatively little has been written about its effectiveness in autoimmune disorders. We have now studied the effect of cladribine in patients with multiple sclerosis (MS). The results of a double-blind crossover investigation suggest that it may prove to be a very effective treatment for this disease.[20,21] Preliminary results obtained in the treatment of other autoimmune disorders suggest a wider applicability of this drug.

MULTIPLE SCLEROSIS

The underlying cause of MS is unknown, but damage to the central nervous system seems to be mediated by autoimmune mechanisms.[10,24] For this reason, immunosuppression, or "immunomodulation," is a rational approach to treatment. Numerous immunosuppressive and immunomodulatory drugs have been administered to patients with MS, but there are few that have shown efficacy in double-blind investigations. Positive findings have, in general, been limited to patients with the relapsing-remitting form of the disease. Efforts at immunomodulation include oral administration of myelin[25] and of copolymer-1.[16] Controlled data on the effectiveness of these experimental therapies are not yet available. High-dose intravenous (IV) methylprednisolone therapy may be effective short-term treatment for acute relapses, but has no effect on chronic progressive disease.[11] Interferon beta-1b (Betaseron®; Berlex Laboratories, Richmond, CA) appeared to be effective in the treatment of relapsing-remitting MS by decreasing the number of relapses, but did not significantly affect the neurologic status of patients[15]; the activity of interferon beta-1b against the chronic progressive form of MS is unknown. Cyclophosphamide has been tested in combination with corticotropin, and the results support a role for immunosuppression in the treatment of chronic progressive MS.[26] A randomized trial of azathioprine in relapsing-remitting MS has confirmed only very modest therapeutic benefit,[12] and low-dose oral methotrexate has shown only slight measurable benefit in chronic progressive MS.[13]

Appraisal of the efficacy of experimental therapeutic agents is particularly difficult in patients with relapsing-remitting MS. We therefore elected to perform our initial trials of the effectiveness of cladribine in MS patients who have the chronic progressive form of the disease. In the preliminary open-label study of four patients who have the chronic progressive form of the disease, we administered cladribine at 0.87 mg/kg/d for 7 days. No toxic effects were encountered. Notably, an increase in the mean corpuscular volume (MCV) of red blood cells occurred and persisted for a prolonged period. The patients appeared to improve clinically, as assessed by an unblinded neurologist.

Study Protocol

Patient selection and randomization. Twenty-four pairs of patients matched by age, sex, and disease severity were initially entered into a double-blind study. Patients paired by the neurologist were randomized by the statistician using random-number tables, so that one patient was assigned to the group initially receiving cladribine

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and the other to the group receiving placebo. Two patients, both initially assigned to cladribine, were lost to follow-up study at 2 and 3 months on study, respectively. Because the loss of these two patients from the initial cladribine arm was not attributed to treatment, two additional patients were recruited as replacements and assigned by the statistician to the cladribine arm. One patient receiving placebo withdrew from the study after 4 months on protocol for reasons related to treatment (ie, lack of stabilization of disease). This patient was not replaced. Four additional patients were lost from the study in the second year for various reasons unrelated to the study. Accordingly, 24 months or longer follow-up study of 21 of 24 patients who had received cladribine in the first year and 22 of 24 patients who had received placebo in the first year was possible.

Cladribine dosage. Patients who had been randomized to the cladribine arm received four monthly 7-day infusions of cladribine, each totaling 0.7 mg/kg. After 1 year, blinding was maintained and these patients received four monthly infusions of saline placebo. The dose of cladribine given to patients who were crossed-over to receive active drug during the second year of the study was reduced, because we encountered more thrombocytopenia than expected in the first year. Thus, those patients who received cladribine during the second year were given one 7-day infusion of cladribine 0.7 mg/kg, followed in the second and third months by 7-day infusions of cladribine each totaling 0.35 mg/kg, and in the fourth month they were given an infusion of placebo. Accordingly, the total dose received by patients who received cladribine in the first year was generally 2.8 mg/kg, and patients who received cladribine in the second year of the study generally received a total dose of 1.4 mg/kg.

However, there were a few deviations from these stated drug dosages. In the original protocol, a total of six monthly doses had been planned, but this was modified to four courses after the study had been initiated, because of a greater-than-expected degree of thrombocytopenia occurring in some patients. One patient had already received five courses before the decision to reduce dosage had been made. One patient received only two courses and two patients received only three courses because of thrombocytopenia. At the beginning of the second phase of the study, five patients who were to have received placebo were given a single dose of cladribine 0.7 mg/kg by error. Separate analysis showed that the response of these patients was not greater than those of the other patients, and the data from these patients have been retained.

Results

Hematological findings and adverse events. Blood cell counts of the patients are summarized in Figs 1 and 2. Although average platelet counts remained normal throughout the study, platelet counts of seven patients receiving the larger dose of cladribine in the first year of the study decreased to less than 100,000 cells/μL. One of these patients, a drug abuser who had taken carbamazepine (Tegretol®; Basel Pharmaceuticals, Summit, NJ) and was ingesting large amounts of phenytoin (Dilantin®; Parke-Davis, Morris Plains, NJ) and very likely other unknown drugs, developed severe thrombocytopenia with platelet counts less than 10,000 cells/μL.[3] This patient remained hematologically normal following her recovery, but her MS began to progress and she committed suicide approximately 2 years after the episode of marrow suppression. In the second year of the study, when patients received only half the dose of cladribine administered in the first year, platelet counts less than 100,000 cells/μL were encountered in only one patient, with the lowest count observed being 83,000 cells/μL. There was marked depletion of CD4 cells and a profound decrease in the CD4:CD8 ratio both in patients who had received a total dose of 2.8 mg/kg in the first year and in those who had received 1.4 mg/kg in the second year (data not shown).

Four patients developed herpes zoster during the course of the study. None of these patients were receiving corticosteroid therapy. All herpetic infections were segmental, mild, and responded rapidly to oral acyclovir. One patient developed fatal, fulminating, newly acquired hepatitis B after her second dose of cladribine. Although it is impossible to be certain about the causal relationship between drug administration and this patient's rapid downhill clinical course, we consider it extremely unlikely that administration of cladribine played a role in this patient's illness. This case and the reasons that we believe her death was unrelated to cladribine therapy are described elsewhere.[21] One patient receiving placebo

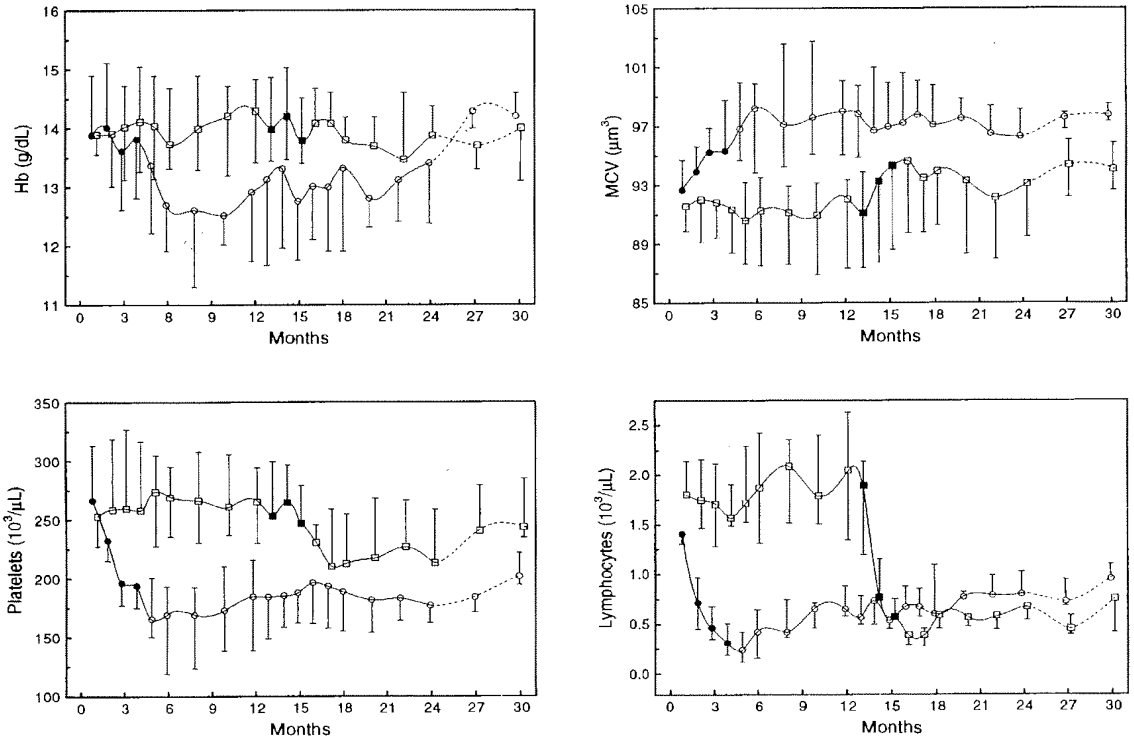

Fig 1. Hemoglobin (Hb), MCV, platelet counts, and lymphocyte counts of patients being treated for MS in a double-blind crossover study. Each point represents the median of the group, and bars represent the 1st and 3rd quartile. Weekly courses of cladribine are depicted by solid symbols. The total amount of cladribine given to patients who received cladribine first (circles) was 2.8 mg/kg. Those receiving cladribine during the second year (squares) received a total dose of 1.4 mg/kg.

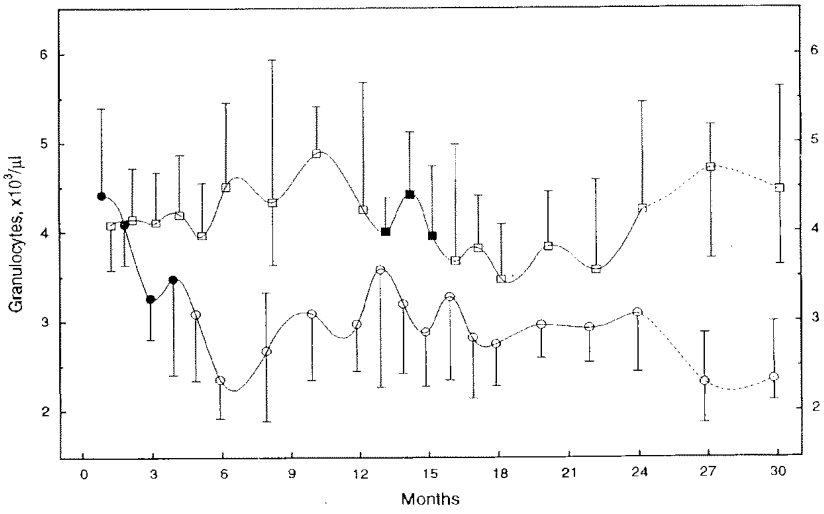

Fig 2. Granulocyte counts of patients being treated for MS in a double-blind crossover study. Each point represents the median of the group, and bars represent 1st and 3rd quartiles. Weekly courses of cladribine depicted by solid symbols. Total amount of cladribine given to patients who received cladribine first (circles) was 2.8 mg/kg. Those receiving cladribine during the 2nd year (squares) received a total dose of 1.4 mg/kg.

during the first year of the study developed a severe *Salmonella* infection with near perforation of the bowel. She responded well to the antibiotic therapy and continued the study.

Clinical neurologic evaluation. Figure 3 depicts the paired differences in Kurtzke Extended Disability Status Scale (EDSS) and Scripps Neurologic Rating Scale (SNRS) scores of patients in the double-blind crossover study. Scores were significantly better (higher for SNRS and lower for EDSS) in cladribine-treated patients than in paired placebo-treated patients until the end of the first year. When cladribine was given at 12 months to patients who had received placebo, the trend was reversed. Average EDSS and SNRS scores for patients receiving cladribine improved

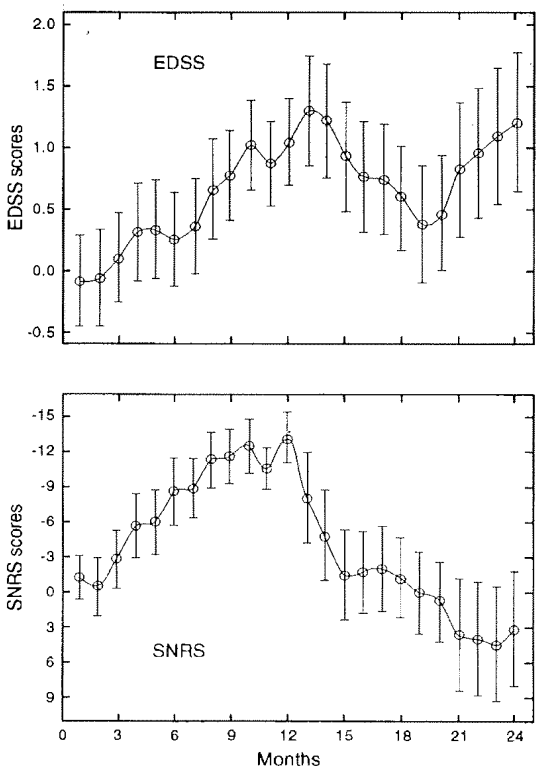

Fig 3. Paired differences in EDSS scores and SNRS scores of patients receiving cladribine and placebo. EDSS and SNRS scores of the member of the pair who was in the group receiving cladribine first were subtracted from the respective scores of the member who received placebo first. Points represent the mean of these differences, and bars represent 1 SEM. Positive differences in the EDSS score and negative differences in the SNRS score represent improvement.

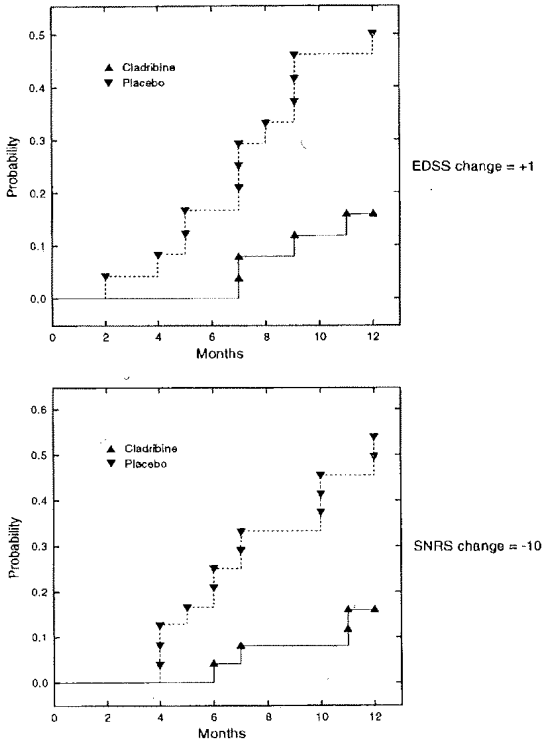

Fig 4. Time-to-failure plots of cladribine- and placebo-treated patients during the first year of the study. Failure is defined as an increase in the EDSS score of ≥ 1 point or a decrease in the SNRS score of ≥ 10 points. At the end of 1 year, the EDSS score of 50% of patients receiving placebo had increased by ≥ 1 point, while only 16% of patients receiving cladribine had experienced such an increase in disability. Similarly, the SNRS score of 54% of patients receiving placebo had decreased by ≥ 10 points, while only 16% of patients receiving cladribine had experienced such a decrease in performance.

modestly during the first year of the study, while the scores for patients receiving placebo continued to deteriorate. The improvement appeared to be well maintained for the 24 months of follow-up study in patients treated with cladribine 2.8 mg/kg. Scores for patients who received placebo in the first year of the study deteriorated during that year, but the lower dose of cladribine they received (1.4 mg/kg) seemed to effectively stabilize the disease in the second year, although the stabilization of disease produced by the lower dose of cladribine may have been of shorter duration than that observed with the larger dose.

Kaplan-Meier time-to-failure plots show that whether failure is defined as a gain of 0.5, 1, or

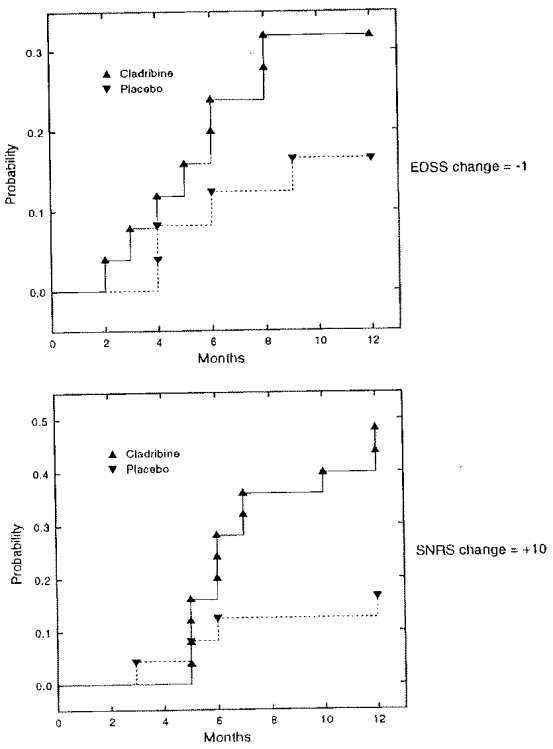

Fig 5. Time-to-improvement plots of cladribine- and placebo-treated patients during the first year of the study. Improvement is defined as a decrease in the EDSS score of ≥ 1 point, or an increase in the SNRS score of ≥ 10 points. At the end of 1 year, the EDSS score of 18% of patients receiving placebo had decreased by ≥ 1 point, while 32% of patients receiving cladribine had experienced such a decrease in disability. Similarly, the SNRS score of 18% of patients receiving placebo had increased by ≥ 10 points, while 45% of patients receiving cladribine had experienced such an increase in performance status.

1.5 points on the EDSS scale or a loss of 5, 10, or 15 points on the SNRS scale, patients receiving cladribine fared better than those who received placebo. Plots showing time to gain of 1 EDSS or loss of 10 SNRS points are shown in Fig 4. Surprisingly, time-to-improvement plots also showed a statistically significant advantage for patients receiving cladribine (Fig 5).

Magnetic resonance imaging findings. Neither nonparametric analysis of variance nor parametric analysis of variance, based on the two-period crossover design, revealed a statistically significant treatment effect on demyelinated volumes from magnetic resonance imaging (Fig 6).

In contrast, a highly significant difference was

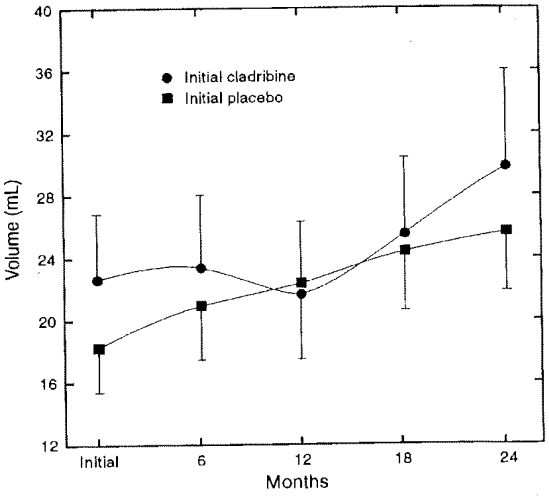

Fig 6. Demyelinated volumes as assessed on magnetic resonance imaging scanning during the course of the study. Changes in demyelinated volumes in patients who received placebo first and those who received cladribine first were not significantly different. Means ± SEMs are plotted.

seen in the enhancing volumes, a measurement of current disease activity. For the purpose of analysis, enhancing volume findings were classified as either having a favorable outcome (disappearance of enhancing volumes or continued absence) or an unfavorable outcome (emergence of or continued presence of enhancing volumes). Results of this analysis are summarized in Table 1.

The Use of Subcutaneously Administered Cladribine

Although cladribine administered by continuous IV infusion was found to be effective in re-

Table 1. Changes in Enhancing Lesion Volumes by Magnetic Resonance Imaging

Time (mo)	Outcome			
	Initial Placebo		Initial Cladribine	
	Favorable	Unfavorable	Favorable	Unfavorable
0 v 6	13	11	18*	6*
6 v 12	12	12	22*	2*
12 v 18	22*	1*	23*	0*
18 v 24	21*	1*	20*	0*

NOTE. A favorable outcome is the continued absence or elimination of enhancing volume; an unfavorable outcome is the emergence or continued presence of enhancing volume.

* Patients had received active drug.

tarding progression of chronic progressive MS, it would be more convenient to implement this type of therapy if the drug could be given by another route. From this point of view, the pharmacologic studies reported by Liliemark et al[19] are particularly important. These investigators showed that the bioavailability of subcutaneously administered cladribine is very similar to that observed when the drug is given by continuous IV infusion. Oral administration, too, was found to be effective, but at twice the dose required to produce similar blood levels when the drug was given IV or subcutaneously. Oral or subcutaneous cladribine was also found to be effective in the treatment of lymphoid leukemias.[17,18]

Although either subcutaneous or oral administration of cladribine to patients with MS would be a more convenient way to treat the disorder, the margin of safety does not seem sufficiently great for us to be comfortable about giving a larger dose by the oral route. It is always possible that some patients might absorb the entire dose and consequently develop marrow suppression. Indeed, the incidence of thrombocytopenia that we observed in patients to whom we gave four courses of cladribine 0.7 mg/kg by IV infusion was sufficiently high that we decided to reduce the dose in further studies in which the drug is administered subcutaneously. The treatment protocol that is under current investigation uses six monthly subcutaneous courses of cladribine, each consisting of 0.07 mg/kg/d for 5 successive days. Because cladribine solutions are currently commercially available only at a concentration of 1 mg/mL, the daily volume that must be given under this protocol is too large to inject at a single site. Therefore, we normally divide each dose into two or three sites, so that not more than 2 mL is injected at a time. The monthly dose of cladribine given under this protocol is only 0.35 mg/kg (half the dose administered in the first year of the double-blind crossover study). The total subcutaneous dose, given as six courses rather than the four courses given in the earlier study, is 2.1 mg/kg (three quarters of the dose administered in the first year of the double-blind crossover study).

We are currently performing double-blind studies of the efficacy of subcutaneous cladribine under the protocol outlined earlier for the treatment of both chronic progressive and relapsing-remitting MS. Because the study has not yet been completed, we have no data on efficacy, but the

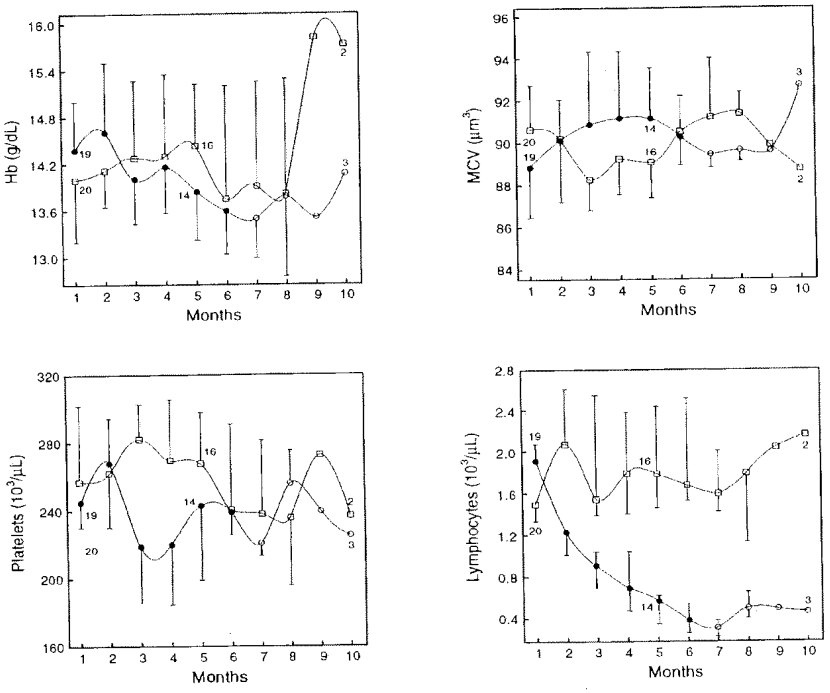

Fig 7. Hemoglobin (Hb), MCV, platelet counts, and lymphocyte counts of patients with relapsing-remitting MS receiving cladribine 0.07 mg/kg/d for 5 days per month for 6 months. Total dose = 2.1 mg/kg. Symbols represent median values, and bars are 1st and 3rd quartiles. Solid symbols indicate months during which cladribine was administered. Since the studies are currently under way, only a few patients have been treated as long as 10 months. The number of patients at 1, 5, and 10 months is shown as a number next to the symbols. At this lower dose-intensity, the rate of change of blood cell counts was more gradual even than that observed in patients receiving a total dose of only 1.4 mg/kg (see Figs 1 and 2).

unblinded statistician (J.K.) can provide laboratory data on the treated and placebo groups without compromising the blinded nature of the study. The effect on blood cell counts of subcutaneously administered cladribine at a dosage of 0.35 mg/kg per course is shown in Fig 7.

OTHER STUDIES

Investigations of the effect of subcutaneously administered cladribine on patients with relapsing-remitting MS have been performed in Poland by Grieb et al[14] and Stelmasiak et al.[22] These investigators arrived independently at the same dose of cladribine, given subcutaneously, that we elected to use in our current studies. Preliminary analysis of the data appears to show a therapeutic effect in this type of MS.

Immune Thrombocytopenic Purpura

Chronic immune thrombocytopenic purpura is an autoimmune disorder manifested by autoantibody-induced platelet destruction. These autoantibodies are most commonly directed against either the platelet glycoprotein (GP) IIb/IIIa and/or the GP Ib/IX complex, and result in accelerated platelet destruction. Immunosuppressive therapy is often useful in the treatment of these patients.[1,23]

Seven female patients with a disease duration ranging from 36 to 276 months were treated with one to three cycles of cladribine. All patients had failed to respond to high-dose corticosteroids, splenectomy, vincristine, danazol, cytoxan, and IV gamma globulin. No patient responded adequately to cladribine.[9] The average platelet count after therapy did not differ significantly from baseline counts before therapy. Only one patient showed any improvement, a prolongation of response to IV IgG.

Other Disorders

Anecdotal evidence indicates that about 50% of patients with autoimmune hemolytic anemia, either primary or secondary to lymphomas, respond to treatment with cladribine.[2] Preliminary results of the treatment of other autoimmune diseases, including rheumatoid arthritis, psoriatic arthritis, and psoriasis, and inflammatory bowel disease have been encouraging, and controlled trials of the efficacy of treatment with cladribine are now underway.

REFERENCES

1. Berchtold P, McMillan R: Therapy of chronic idiopathic thrombocytopenic purpura in adults. Blood 74:2309-2317, 1989
2. Beutler E: New chemotherapeutic agent: 2-Chlorodeoxyadenosine. Semin Hematol 31:40-45, 1994
3. Beutler E, Koziol J, McMillan R, et al: Marrow suppression produced by repeated doses of cladribine. Acta Haematol (Basel) 91:10-15, 1994
4. Carson DA, Kaye J, Matsumoto S, et al: Biochemical basis for the enhanced toxicity of deoxyribonucleosides toward malignant human T cell lines. Proc Natl Acad Sci USA 76: 2430-2433, 1979
5. Carson DA, Kaye J, Wasson DB: The potential importance of soluble deoxynucleotidase activity in mediating deoxyadenosine toxicity in human lymphoblasts. J Immunol 126:348-352, 1981
6. Carson DA, Wasson DB, Beutler E: Antileukemic and immunosuppressive activity of 2-chloro-2'-deoxyadenosine. Proc Natl Acad Sci USA 81:2232-2236, 1984
7. Carson DA, Wasson DB, Kaye J, et al: Deoxycytidine kinase–mediated toxicity of deoxyadenosine analogs toward malignant human lymphoblasts in vitro and toward murine L1210 leukemia in vivo. Proc Natl Acad Sci USA 77:6865-6869, 1980
8. Carson DA, Wasson DB, Taetle R, et al: Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. Blood 62:737-743, 1983
9. Figueroa M, McMillan R: 2-Chlorodeoxyadenosine in the treatment of chronic refractory immune thrombocytopenic purpura. Blood 81:3484-3485, 1993
10. Ford HC: Multiple sclerosis: A survey of alternative hypotheses concerning aetiology, pathogenesis and predisposing factors. Med Hypotheses 24:201-216, 1987
11. Francis DA: The current therapy of multiple sclerosis. J Clin Pharmacol Ther 18:77-84, 1993
12. Goodkin DE, Bailly RC, Teetzen ML, et al: The efficacy of azathioprene in relapsing-remitting multiple sclerosis. Neurology 41:20-25, 1991
13. Goodkin DE, Rudick RA, Vanderbrug Medendorp S, et al: Low-dose (7.5 mg) oral methotrexate reduces the rate of progression in chronic progressive multiple sclerosis. Ann Neurol 37:30-40, 1995
14. Grieb P, Ryba M, Stelmasiak Z, et al: Cladribine treatment of multiple sclerosis. Lancet 344:538, 1994 (letter)
15. Jacobs L, Goodkin DE, Rudick RA, et al: Advances in specific therapy for multiple sclerosis. Curr Opin Neurol 7:250-254, 1994
16. Johnson KP: Experimental therapy of relapsing-remitting multiple sclerosis with copolymer-1. Ann Neurol 36: S115-S117, 1994
17. Juliusson G, Liliemark J: Complete remission of B-cell chronic lymphocytic leukaemia after oral cladribine. Lancet 341:54, 1993 (letter)
18. Juliusson G, Heldal D, Hippe E, et al: Subcutaneous injections of 2-chloro-deoxyadenosine (2-CdA) for symptomatic hairy cell leukemia. J Clin Oncol 13:989-995, 1995

19. Liliemark J, Albertioni F, Hassan M, et al: On the bioavailability of oral and subcutaneous 2-chloro-2′-deoxyadenosine in humans: Alternative routes of administration. J Clin Oncol 10:1514-1518, 1992

20. Sipe J, Koziol J, Romine J, et al: Cladribine treatment of multiple sclerosis. Lancet 344:537-538, 1994 (reply)

21. Sipe JC, Romine JS, Koziol JA, et al: Cladribine in treatment of chronic progressive multiple sclerosis. Lancet 344:9-13, 1994

22. Stelmasiak C, Solski J, Nowicki J, et al: Short-term effects of 2-chlorodeoxyadenosine (2-CdA) in remitting-relapsing multiple sclerosis (MS). J Neurol 241:S90, 1994 (suppl 1, abstr)

23. Warkentin TE, Kelton JG: Current concepts in the treatment of immune thrombocytopenia. Drugs 40:531-542, 1990

24. Weiner HL, Hafler DA: Immunotherapy in multiple sclerosis. Ann Neurol 23:211-222, 1988

25. Weiner HL, Mackin GA, Matsui M, et al: Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis. Science 259:1321-1324, 1993

26. Weiner HL, Mackin GA, Orav EJ, et al: Intermittent cyclophosphamide pulse therapy in progressive multiple sclerosis: Final report of the Northeast Cooperative Multiple Sclerosis Treatment Group. Neurology 43:910-918, 1993

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The treatment of chronic progressive multiple sclerosis with cladribine

(immunosuppression/double-blind study/crossover/2-chlorodeoxyadenosine/deoxypurine)

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ABSTRACT **A 2-year, placebo-controlled, double-blind, crossover study was started in 1992 to evaluate cladribine, an immunosuppressive drug, in the treatment of chronic progressive multiple sclerosis. In the first year patients were given cladribine 0.10 mg/kg per day for 7 days as four monthly courses for a total of 2.8 mg/kg or placebo. During the second year patients treated with placebo during the first year were given i.v. infusions of 0.10 mg, 0.05 mg, and 0.05 mg of cladribine per kg of body weight per day for 7 consecutive days in three successive monthly courses, for a total dose of 1.4 mg/kg. Patients who had been treated previously with cladribine were crossed over to placebo. Analysis of the results revealed a favorable influence on the neurological performance scores, both in the Kurtze extended disability status and the Scripps neurological rating scale, and on MRI findings in patients treated with cladribine. In the first year the most striking finding was that while clinical deterioration continued in the placebo-treated patients, the condition of patients who received cladribine stabilized or even improved slightly. Toxicity and therapeutic response were dose-related.**

Although the underlying cause of multiple sclerosis (MS) remains a mystery, considerable evidence exists that damage to the central nervous system is mediated by immunopathologic mechanisms (1, 2). For this reason, immunosuppression is a rational approach to treatment of this disorder.

2-Chlorodeoxyadenosine (2-CdA; cladribine; Leustatin) is an adenosine deaminase-resistant purine nucleoside, designed by Carson *et al.* (3–5) to simulate the immunodeficiency state of hereditary adenosine deaminase deficiency by causing the accumulation of deoxynucleotides in lymphocytes. This simple compound has been widely used for the treatment of lymphoid malignancies (6) and has a very favorable toxicity profile relative to other lymphocytolytic drugs.

Since there is no satisfactory treatment for chronic progressive MS, the use of cladribine was considered because of its relatively low toxicity and the long-lasting lymphopenia that it produces. Open-label feasibility studies were begun in 1990 with a small number of patients. The results in terms of both apparent benefit on neurological performance and lack of toxicity were favorable and encouraged us to proceed with a larger 2-yr placebo-controlled crossover study to further explore issues of safety and therapeutic effect. The first-year results of this study showed a positive effect (7). We now report observations from the entire 2 yr of this double-blind study and an additional 6-mo unblinded follow-up.

METHODS

Patient Selection. The study subjects were 51 patients with clinically definite or laboratory-supported definite chronic progressive MS (8) for >2 yr. The patients had been followed at Scripps Clinic by the neurology group for periods of from 6 mo to 20 yr. The study plan and risks and potential benefits were explained to each patient in detail, and all patients gave informed consent to participate in the investigations, which were performed under investigator-initiated INDs no. 29,111 and no. 93,777 from the Food and Drug Administration.

Patient characteristics are summarized in Table 1.

Study Design. *Drug dosage.* A phase III double-blind crossover study was started in January of 1992. In the first year of this study patients on the cladribine arm were given four monthly 7-day courses of 0.10 mg of cladribine per kg per day (0.7 mg/kg per course) for a total of four courses (total dose = 2.8 mg of cladribine per kg), except as noted below. During the second year blinding was maintained, and the patients who had received placebo were given active drug, but at one-half the total dose that had been administered during the first year. The four infusions given to these patients were divided so that the first consisted of 0.7 mg of cladribine per kg, the second and third each consisted of 0.35 mg of cladribine per kg of body weight, and the fourth consisted of saline placebo (total dose = 1.4 mg of cladribine per kg). The patients who had originally received cladribine were given four monthly saline placebo infusions from the beginning of the second year.

Cladribine or placebo was administered monthly on an outpatient basis by 7-day i.v. infusions through a central venous catheter using a portable infusion pump. Blood counts and chemistry panels were done before each infusion, and the counts, reviewed by an unblinded hematologist/internist, had direct contact with only four of the patients when a medical problem required internal medicine consultation.

Throughout the study, patients, neurologists, nurses, and the neuroradiologist were unaware of the treatment assigned to each patient. Cladribine causes no symptoms on infusion and cannot be distinguished from placebo by patients or professional staff. Drug was withheld on occasions when blood counts did not meet the safety standards that had been established; this occurred on four occasions in patients receiving active drug and in two patients receiving placebo. Corticosteroid therapy was permitted when the examining neurologist considered them necessary for treatment of the patient. Two patients received corticosteroids during the study; one patient required one course during placebo administration, and another patient required two courses during cladribine therapy.

Sample size and patient compliance. On the basis of the results of the open label study, we had estimated that a sample size of 22 pairs of patients would be sufficient to detect a 15% improvement in the Scripps neurological rating scale (SNRS)

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Abbreviations: MS, multiple sclerosis; SNRS, Scripps neurological rating scale; EDSS, Kurtze extended disability status.

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Table 1. Demographic characteristics of patients at study entry

Characteristic	Initial placebo	Initial cladribine
Sex (F/M)	16/8	18/9
Race (White/other)	22/2	27/0
Mean age, yr (range)	42.5 (21–54)	43.4 (28–54)
Mean duration of clinical symptoms of MS, yr (range)	10.5 (2–31)	12.7 (2–24)

while on cladribine, compared with no improvement while receiving placebo, with a statistical power of 0.90, using a one-sided test at an α level of 0.05. Initially 24 pairs of patients were matched by age, sex, and disease severity. Each matched pair was randomized by the statistician (J.A.K.) using random number tables so that one patient was assigned to the group initially receiving cladribine and the other to the group receiving placebo. One additional individual, for whom no suitable match was identified, was started on the cladribine arm; this individual left the study after 8 mo on the protocol. Two patients, both initially assigned to cladribine, were lost to follow-up at 2 and 3 mo on the study, respectively. Because the loss of these two patients on the initial cladribine arm was not attributed to treatment, we recruited two additional patients, appropriately matched by the "blinded" neurologists (J.C.S. and J.S.R.) and assigned by the statistician to cladribine, as replacements. One patient receiving placebo withdrew from the study after 4 mo on the protocol for reasons related to treatment (i.e., lack of stabilization of disease); this patient was not replaced. The analyses reported are based on the experience of the 24 matched pairs of patients and exclude the three patients initially on cladribine who did not complete a full year of the study, as described above. Four additional patients were lost from the study in the second year for various reasons unrelated to the study. Accordingly, 24-mo or longer follow-up of 21 of the 24 patients who had received cladribine in the first year and 22 of the 24 patients who had received placebo in the first year was possible.

Deviations from the original protocol. There were a few deviations from the stated drug dosages. In the original protocol a total of six monthly doses had been planned, but this was modified to four courses after the study had been initiated because of a greater than expected decline in the platelet count in some patients. One patient received five courses before the decision to reduce dosage had been made. One patient received only two courses, and two patients received only three courses because of thrombocytopenia. At the beginning of the second phase of the study, five patients who were to have received placebo were given a single 0.7 mg/kg of infusion cladribine by error. Separate analysis showed that the response of these patients was not greater than those of the other patients, and the data of these patients have been retained.

Observations and Data Analysis. *Neurologic evaluation.* Patients were evaluated monthly by means of the Kurtzke extended disability status scale (EDSS) (9) and with the SNRS (10). MRI brain scans with contrast enhancement were performed in these patients before treatment and at 6-mo intervals.

To assess inter-rater variability 20 patients were independently assessed by each examiner (J.S.R., J.C.S.) on the same day. Interrater agreement (11) on each scoring instrument was quite high: the weighted κ coefficient of agreement was 0.976 for the EDSS scores and 0.828 for the SNRS scores. Interrater agreement, defined as a difference of $\leq$1.0 EDSS points, reached 100% for all sets of examinations. This result compared favorably with results reported in other clinical trials of investigative therapeutic agents in MS (12). In comparison, interrater agreement on the SNRS was 85%, with agreement defined as a difference of $\leq$10 SNRS points. In a separate evaluation 18 patients were assessed by the same examiner twice on the same day, the period between examinations ranging from 135 min to 240 min. Intra-rater agreement on the EDSS was perfect with both examiners, and weighed κ coefficients of agreement between the two SNRS scores were 0.978 (J.S.R.) and 0.998 (J.C.S.).

Hematologic evaluation. Monthly blood counts were obtained during drug administration. Less frequent counts were obtained thereafter. Lymphocyte subset evaluation was done monthly during the course of drug administration and less frequently thereafter.

MRI. MRI was performed on a 1.5-T General Electric Signa scanner. T_2 and proton density-weighted images were obtained by using a conventional spin-echo sequence with repetition times of 2500 msec and echo-delay times of 30 and 90 msec. Axial scans of 3-mm thickness and 0 interslice gap were obtained $\approx$10 min after gadopentetate dimeglumine (Magnevist, Berlex Laboratories) injection to assure optimal time for transmigration of the contrast agent across the blood–brain barrier.

Statistical Methods. The SNRS was designated as the primary outcome parameter. Summary statistics are reported as mean and range or SEM. Analyses of the neurological scores and MRI findings were undertaken with parametric methods appropriate for two-period repeated-measurements crossover designs (13) as well as a nonparametric repeated-measures ANOVA technique (14). Last available observations were carried forward for patients who had completed at least 18 mo of the study. Similar analyses were done in which these data remained missing and in which they were modeled under the representation that they were missing at random; these analyses yielded similar results to those reported here. Kaplan–Meier curves and log–rank statistics were also used to compare neurological rating-scale outcomes between the two treatment groups during the first year of the study. Two-sided P values are reported throughout.

RESULTS

Neurologic Findings. *Clinical performance scores.* Fig. 1 depicts the EDSS and SNRS scores of the patients. The average EDSS and SNRS scores of patients receiving cladribine improved modestly during the first year of the study, whereas the scores of patients with placebo continued to deteriorate. The improvement in SNRS scores appeared to peak at $\approx$18 mo and be well maintained for the 24 mo of follow-up in the patients treated with 2.8 mg of cladribine per kg, even though they received no active drug after the first 4 mo of the study. After 24 mo, in unblinded observations, fairly rapid deterioration was documented. While the scores of the patients who received placebo in the first year of the study deteriorated during that year, the lower dose of cladribine they received (1.4 mg/kg) also seemed to be effective in stabilizing their disease, albeit for a shorter time period, with peak improvement at $\approx$8 mo after treatment initiation. Inspection of the curves thus suggests that the stabilization of disease produced by the lower dose of cladribine may be of shorter duration than that seen with the larger dose and that a rebound worsening of disease may occur between 24 and 30 mo after initiation of therapy with the higher dose. ANOVA based on the two-period crossover design with absolute changes in EDSS and SNRS as end points revealed no significant carryover effects between subjects or period effects within subjects, but highly significant treatment effects: the F-statistics for assessing treatment effects with subjects were $F_{1,44} = 10.19$, $P = .0026$ for EDSS and $F_{1,44} = 23.46$, $P < 0.0001$ for SNRS.

Kaplan–Meier time-to-failure plots show that whether failure is defined as a gain of 0.5, 1, or 1.5 points on the EDSS scale or loss of 5, 10, or 15 points on the SNRS scale, patients receiving cladribine fared better than those who received placebo in the first study year. Plots showing time-to-gain of 1.0

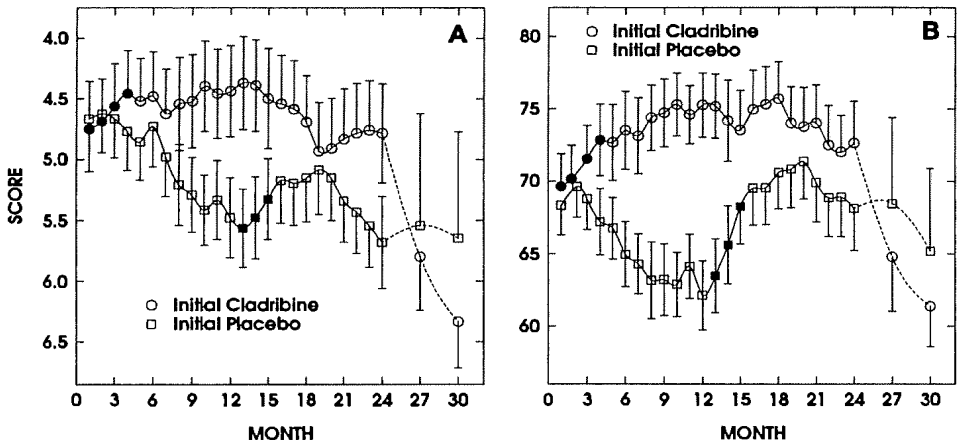

FIG. 1. The EDSS (*A*) and SNRS (*B*) of 48 patients treated with cladribine in a double-blind crossover study. The solid symbols indicate months during which drug was administered. The group of patients denoted by circles was given 0.7 mg of cladribine per kg of body weight in a 7-day infusion during 4 mo. The group that received placebo first (□) was given 0.7 mg of cladribine per kg of body weight as a first infusion (first solid square) and two subsequent 7-day infusions of 0.35 mg per kg of body weight (second and third solid square). The fourth infusion in these patients was placebo, as denoted by the open square. Bars represent 1 SEM. Dashed lines represent changes occurring after the study had been unblinded.

EDSS or loss of 10 SNRS points are shown in Fig. 2. Surprisingly, time-to-improvement plots (data not shown) also showed a statistically significant advantage for patients receiving cladribine.

Log–rank statistics were used to compare times-to-failure between the two treatment groups of the first year of the study, where failure was defined as a gain of 1 or 1.5 points on the EDSS or a loss of 10 or 15 points on the SNRS relative to baseline value. Each of these statistics was highly significant (ΔEDSS = 1.0:L = 6.313, P = 0.012; ΔEDSS = 1.5:L = 5.254, P = 0.024; ΔSNRS = −10:L = 8.299, P = 0.004; ΔSNRS = −15:L = 6.800, P = 0.009), indicating that patients receiving cladribine fared better than those who received placebo.

MRI findings. Neither nonparametric ANOVA nor parametric ANOVA based on the two-period crossover design revealed a significant treatment effect on demyelinated volumes on MRI (data not shown). In contrast, a highly significant difference was seen in the enhancing volumes, a measurement of current disease activity (15). For analysis, enhancing volume findings were dichotomized as being either present or absent; the results of this analysis are summarized in Table 2. At the end of 1 yr, 12 patients in the group given placebo were scored as having no enhancing lesions: 9 because they had no enhancing lesions throughout the period, and 3 because they had lesions that disappeared. However, the other 12 patients were scored as having lesions: 10 because enhancing lesions persisted throughout the 12-mo period, and 2 because they developed new enhancing lesions. In contrast, at the end of the first year, 22 of the patients who had received cladribine were classified as having no lesions: 11 patients had had no enhancing volumes at baseline, and 11 had enhancing lesions that disappeared. Only two individuals in this group had lesions: one had continuing enhancing lesions, and one developed new enhancing lesions where there had been none (P < 0.001, McNemar's test).

Further analysis was made of patients after the crossover of treatments at 12 mo. Twenty-two of the patients in the group that received placebo first and who were then treated with 1.4 mg of cladribine per kg of body weight were evaluated at 24 mo. Eleven had no lesions at both 12 and 24 mo, one had lesions at both 12 and 24 mo, and 10 had lesions at 12 mo but none at 24 mo (P < 0.001, McNemar test). From this crossover analysis, there is clear evidence that the reduced dose of cladribine received by the initial placebo group during the second year on the study significantly reduced the occurrence of enhancing lesions on MRI scans. Among the 20 patients in the group that received cladribine first who were evaluated at

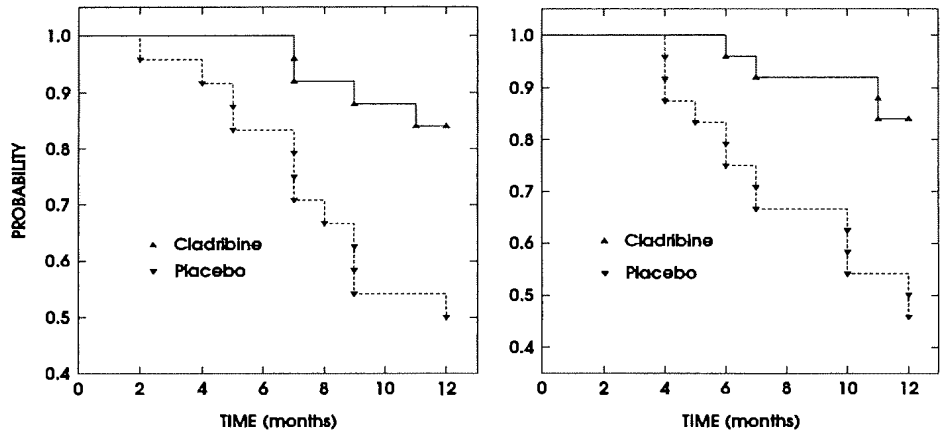

FIG. 2. Kaplan–Meier time-to-failure plots of patients in the double-blind crossover study. (*Left*) Time-to-increase of EDSS score by 1.0 point. (*Right*) Time-to-failure as defined by a decrease of SNRS score by 10 points. The difference in time-to-failure estimated by logarithmic–rank statistics was highly significant (EDSS, P = 0.012; SNRS, P = 0.004).

Table 2. Presence of enhancing lesions by MRI over the study

	Outcome			
	Initial placebo		Initial cladribine	
Time	Lesions absent	Lesions present	Lesions absent	Lesions present
Baseline	11	13	12	12
6 mo	13	11	18	6
12 mo	12	12	22	2
18 mo	22	1	23	0
24 mo	21	1	20	0

24 mo, 19 had no lesions both at 12 and at 24 mo, and one had lesions at 12 but none at 24 mo. Thus, the treatment effect found in the group that received cladribine in the first year seemed to carry over for an additional 12 mo.

Toxicity and adverse events. The average platelet counts remained normal throughout the study, but the platelet counts of seven patients receiving the larger cladribine dose in the first year of the study fell to $<100{,}000/\mu l$, and one of these, a patient who had taken carbamazepine (Tegretol) and who was ingesting large amounts of phenytoin (Dilantin), developed severe thrombocytopenia with platelet counts $<10{,}000/\mu l$. The effect of cladribine on the blood counts of these patients has been reported in detail elsewhere (16). In the second year of the study, when patients received only one-half of the dose of cladribine administered in the first year, platelet counts of $<100{,}000/\mu l$ were encountered in only one patient, the lowest count observed being $83{,}000/\mu l$. Changes in lymphocyte subsets are depicted in Fig. 3. There was marked depletion of CD4 cells with both the high- and low-dose schedule.

Six patients developed herpes zoster, one of them only after retreatment with cladribine under another protocol. All of these infections were segmental, mild, and responded rapidly to oral acyclovir. One patient developed fatal, fulminating, newly acquired hepatitis B immediately after her second dose of cladribine. As discussed elsewhere (7), it seems unlikely that the administration of cladribine played a role in this patient's illness. One patient developed a severe *Salmonella* infection with near-perforation of the bowel while receiving placebo during the first study year. She responded well to antibiotic therapy and continued the study.

DISCUSSION

Although numerous immunosuppressive and immunomodulatory drugs have been given to patients with MS, conventional immunosuppression has not demonstrated sufficient promise to be considered as a routine treatment for the chronic progressive form of MS (17–19). Why then, might another immunosuppressive agent produce a greater effect? The suppression of CD4 cells and sparing of CD8 cells that was observed with cladribine administration is much greater than that observed with other immunosuppressive agents. Treatment of MS patients for a year with chlorambucil (18) and cyclophosphamide (20) each produced a relatively transient 2-fold decline in the CD4/CD8 ratio. In contrast, treatment with cladribine for only a few months produced an ≈4-fold decrease in this ratio, and the effect was sustained for many months after a 4-mo course of the drug.

Because of its selective and prolonged effect on T cells, cladribine appeared to be a reasonable candidate drug for MS treatment. Our studies clearly show that cladribine retards the progression of neurologic impairment of patients with chronic

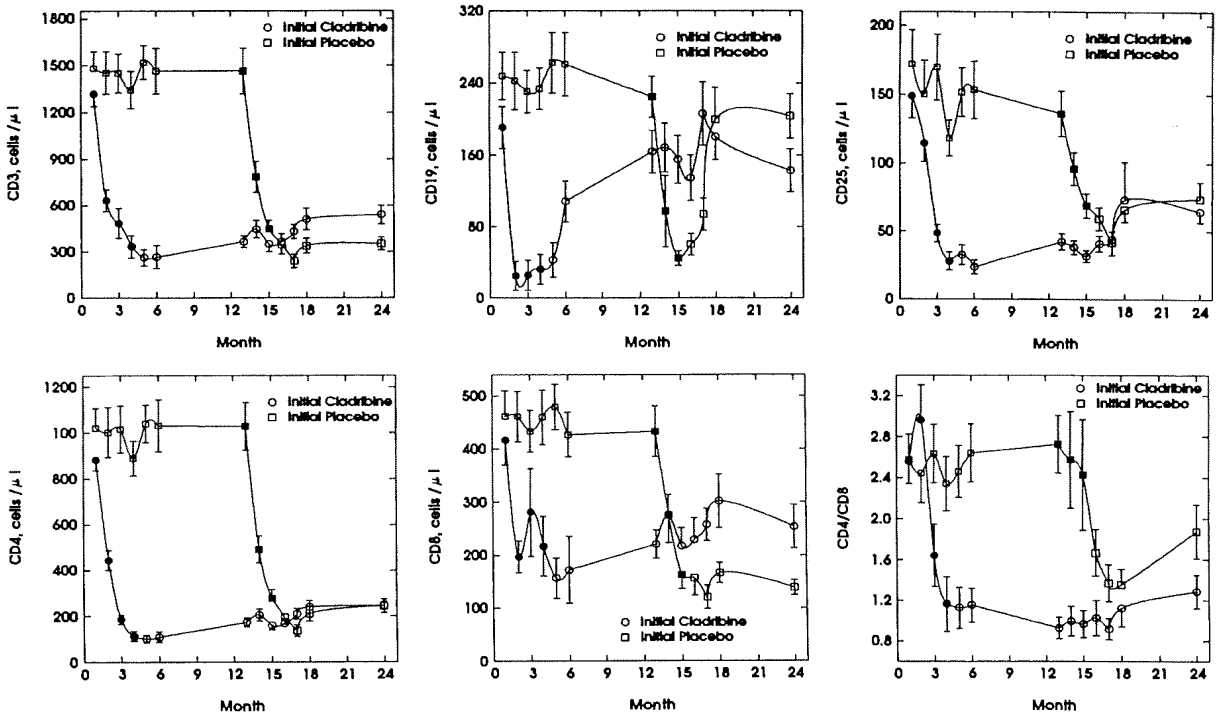

FIG. 3. Changes in lymphocyte subsets in patients. Symbols and drug dosages are as in Fig. 1. The decline of T lymphocytes (CD3) was more marked than that of B lymphocytes. A rapid and profound fall in the inducer/helper (CD4) T lymphocytes was accompanied by a more modest and less sustained decline in the cytotoxic/suppressor (CD8) T lymphocytes. Accordingly, there was an ≈4-fold decline in the CD4/CD8 ratio. The number of activated T and B lymphocytes (CD25) fell ≈5-fold and remained at subnormal levels for the 14 mo after cessation of drug administration. The slight dip in some of the lymphocytes in the group that received cladribine first is due to the erroneous administration of a single dose of drug to a few of the patients in that group on month 13.

progressive MS. The effect is of sufficient magnitude that highly statistically significant results could be obtained by the study of only 24 pairs of patients in a double-blind study. The smaller effects produced by other drugs have required that much larger groups of patients be investigated. At a total dose of 2.8 mg/kg given over a period of 4 mo, the stabilization of disease appears quite durable.

As for other cytotoxic agents such as methotrexate and cyclophosphamide, marrow suppression is a known side-effect of cladribine (6, 21). In the present study occasional patients developed significant thrombocytopenia at higher drug dosages; one patient developed severe but reversible marrow suppression. The lower dose of cladribine used in the second arm of the present study appears to provide a larger margin of safety. From this point of view, we were encouraged by the therapeutic effect of 1.4 mg/kg of drug given over a 3-mo period, a dose that appeared to be very well tolerated. The therapeutic effect of the lower dose appeared to be less durable than that observed at higher doses, this dosage–response relationship lending further weight to the validity of our results.

The usefulness of MRI as a measure of clinical activity and potentially as an objective means for assessing response to therapy has recently been documented (19). In our studies we observed marked improvement in the appearance and disappearance of lesions visualized on MRI after gadolinium enhancement. These highly statistically significant changes persisted for the full 2 yr of observation in the patients who had received 4 mo of therapy in the first arm of the study and were seen in patients receiving the low cladribine dose as well as in those receiving the high dose.

Treatment with immunosuppressive drug is not without risk. The occurrence of herpes zoster in six of the patients is surely a manifestation of their immunosuppressed state, but no other opportunistic infections attributed to cladribine therapy were encountered, in spite of the fact that the CD4 cell count remained low for long time periods. Many immunosuppressive drugs are also myelosuppressive, and cladribine does exhibit a dose-dependent effect on hematopoietic stem cells. In the current study thrombocytopenia was noted in some patients receiving higher doses of the drug, and severe, transient marrow suppression occurred in one patient who was also ingesting other potentially myelosuppressive drugs (16). Although the short-term risks of cladribine seem acceptable for patients with severely progressive disease, little can be written about the long-term risk. We began using this drug, primarily in patients with malignant disorders, in 1981. Most patients treated before 1987 had end-stage malignant disease, and only a few are alive today. In the past 8 yr, however, many patients with hairy cell leukemia and with relatively early-stage lymphomas have been treated with the drug. No long-term toxic results have been documented, but since the drug is incorporated into DNA (3), the possibility of malignancies occurring long after administration of this purine analogue cannot be dismissed.

Cladribine was given by continuous i.v. infusion on an outpatient basis to all of the patients reported here. This route of infusion was used because the safety and efficacy of the drug given by other routes had not been established at the time this study was initiated. We now recognize that the drug may be given by the much more convenient subcutaneous route without causing local irritation and with pharmacokinetic (22) and therapeutic (23) results that appear entirely equivalent to the i.v. route. We have designed a study of the effect in both chronic progressive and relapsing/remitting MS of subcutaneously administered cladribine, 0.07 mg/kg per day given as six monthly courses each consisting of five daily injections. It is notable, that this method of administration has recently been used in MS patients by Grieb *et al.* (24) who report preliminary data suggesting beneficial results in the treatment of relapsing/remitting disease with cladribine.

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1. Ford, H. C. (1987) *Med. Hypotheses* **24,** 201–216.
2. Weiner, H. L. & Hafler, D. A. (1988) *Ann. Neurol.* **23,** 211–222.
3. Carson, D. A., Wasson, D. B., Taetle, R. & Yu, A. (1983) *Blood* **62,** 737–743.
4. Carson, D. A., Wasson, D. B. & Beutler, E. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2232–2236.
5. Carson, D. A., Wasson, D. B., Kaye, J., Ullman, B., Martin, D. W., Jr., Robins, R. K. & Montgomery, J. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6865–6869.
6. Beutler, E. (1994) *Semin. Hematol.* **31,** 40–45.
7. Sipe, J. C., Romine, J. S., Koziol, J. A., McMillan, R., Zyroff, J. & Beutler, E. (1994) *Lancet* **344,** 9–13.
8. Poser, C. M., Paty, D. W., Scheinberg, L., McDonald, W. I., Davis, F. A., Ebers, G. C., Johnson, K. P., Sibley, W. A., Silberberg, D. H. & Tourtellotte, W. W. (1983) *Ann. Neurol.* **13,** 227–231.
9. Kurtzke, J. F. (1983) *Neurology* **33,** 1444–1452.
10. Sipe, J. C., Knobler, R. L., Braheny, S. L., Rice, G. P., Panitch, H. S. & Oldstone, M. B. (1984) *Neurology* **34,** 1368–1372.
11. Cohen, J. (1968) *Psychol. Bull.* **70,** 213–220.
12. Goodkin, D. E., Cookfair, D., Wende, K., Bourdette, D., Pullicino, P., Scherokman, B. & Whitham, R. (1992) *Neurology* **42,** 859–863.
13. Jones, B. & Kenward, M. G. (1989) *Design and Analysis of Cross-Over Trials* (Chapman & Hall, London).
14. Koziol, J. A. & Maxwell, D. A. (1983) *Stat. Med.* **1,** 83–89.
15. Miller, D. H., Barkhof, F., Berry, I., Kappos, L., Scotti, G. & Thompson, A. J. (1991) *J. Neurol. Neurosurg. Psychiatr.* **54,** 683–688.
16. Beutler, E., Koziol, J., McMillan, R., Sipe, J. C., Romine, J. S. & Carrera, C. J. (1994) *Acta Haematol. (Basel)* **91,** 10–15.
17. Goodkin, D. E., Bailly, R. C., Teetzen, M. L., Hertsgaard, D. & Beatty, W. W. (1991) *Neurology* **41,** 20–25.
18. Chiappelli, F., Myers, L. W., Ellison, G. W., Liao, D. & Fahey, J. L. (1991) *Int. J. Immunopharmacol.* **5,** 455–461.
19. Khoury, S. J., Guttmann, C. R., Orav, E. J., Hohol, M. J., Ahn, S. S., Hsu, L., Kikinis, R., Mackin, G. A., Jolesz, F. A. & Weiner, H. L. (1994) *Neurology* **11,** 2120–2124.
20. Moody, D. J., Fahey, J. L., Grable, E., Ellison, G. W. & Myers, L. W. (1987) *J. Neuroimmunol.* **14,** 175–182.
21. Beutler, E. (1992) *Lancet* **340,** 952–956.
22. Liliemark, J., Albertioni, F., Hassan, M. & Juliusson, G. (1992) *J. Clin. Oncol.* **10,** 1514–1518.
23. Juliusson, G., Liliemark, J., Hippe, E., Blichfeldt, P., Wallman, K., Hagberg, H., Stolt, C.-M., Bergheim, J., Winquist, I., Hedenus, M., Carlsson, M., Velin, L., Hellquist, L., Kristofferson, R., Ly, B. E. & Väärt, J. (1992) *Blood* **80,** Suppl. 1, 359 (abstr.).
24. Grieb, P., Ryba, M., Stelmasiak, Z., Nowicki, J., Solski, J. & Jakubowska, B. (1994) *Lancet* **344,** 538.

(Neurology 1995;45:2173–2177). This IFNγ-secreting cell surge has been linked to an increased risk of exacerbations in RRMS patients following initiation of therapy with IFNβ-1b.

DESIGN/METHODS: We carried out a retrospective study by reviewing the medical records (whenever possible) and interviewing 192 RRMS patients. The study examined a 90 day period from the start of treatment with IFNβ-1b as well as a "control period" for each patient. The control period for each patient was defined as the 90 days immediately prior to the start of treatment with IFNβ-1b. Patient diagnosis and clinical course was confirmed from a patient registry established to enroll MS patients for treatment with IFNβ-1b. Details regarding symptoms, exacerbations, number and duration of treatment with steroids, and change in medication were carefully reviewed. Patient age ranged from 23 to 64 years (mean: 38.4). One hundred and twenty six patients out of 192 (66.1%) were women. Duration of disease ranged from 8 months to 20 years (mean: 7.8 years). Duration of treatment with IFNβ-1b ranged from 6 to 30 months (mean: 16.8 months). Seventy three patients (38.1%) had discontinued treatment with IFNβ-1b at the time of this study.

RESULTS: Twenty out of 192 (10.4%) patients did not have exacerbations in the control period, but experienced exacerbations in the first 90 days of treatment with IFNβ-1b. None of these patients had received steroids in the control period. Forty one out of 192 (21.4%) patients had exacerbations during the control period but none in the 90 days after the start of treatment with IFNβ-1b. Twenty six of these 41 patients received steroids to treat the relapses. Fourteen out of 192 (7.3%) patients had relapses during the control as well as the post-treatment 90 day periods. One hundred and seventeen out of 192 (60.9%) patients did not experience exacerbations in the control or post-treatment 90 day periods. Initiation of treatment with IFNβ-1b did not cause an increased frequency of exacerbations within 90 days of beginning treatment . Infact treatment with IFNβ-1b was associated with a significant decrease in the frequency of exacerbations within 90 days of beginning treatment ($p<0.02$, by McNemar's test).

CONCLUSION: Although retrospective, our study did not demonstrate an increased rate of exacerbations observed in the first 90 days of starting treatment with IFNβ-1b. Therefore, it is possible that the IFNγ-secreting cell surge seen following initiation of treatment with IFNβ-1b, may not be clinically significant.

P03.068

Characteristics of Relapsing-Remitting Multiple Sclerosis Patients as Possible Indicators of Response to Interferon Beta-1b (IFN BETA-1b) Treatment

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OBJECTIVE: In this post hoc analysis, we attempt to correlate baseline and on study characteristics of relapsing-remitting multiple sclerosis patients with IFN beta-1b treatment outcome measures.

BACKGROUND: IFN beta-1b is the first approved therapy for MS. It reduced the frequency and severity of clinical relapses and the amount of total disease activity as measured in serial MRI scans in a pivotal trial that included 372 patients with relapsing-remitting MS . It also reduced the number of new or enlarging MRI lesions in a frequently scanned subgroup of patients.

DESIGN/METHODS: This analysis was based on the complete 5-year dataset from the IFN beta-1b double-blinded, placebo-controlled, randomized study that was completed in 1993. Patients who received 8 mIU were analyzed. Several patient baseline characteristics were examined with respect to: (1) annualized relapse rate, (2) percent change in MRI lesion area, and (3) time to a 1.0 confirmed increase in EDSS score.

RESULTS: We identify no reliable predictors of patient response to IFN beta-1b therapy. Similarly, there is no difference in the baseline characteristics of patients whom we arbitrarily define as "responders" and "non-responders". IFN beta-1b appears to be effective, regardless of the age at disease onset, prior relapse rate, disease duration, baseline Kurtzke's EDSS score, FS scores, baseline Scripps score, or gender. IFN beta-1b-associated adverse events cannot predict treatment outcome. Patients who experience a relapse in the first month on study have a poorer outcome.

CONCLUSIONS: The results of this study suggest that MS patients with a wide range of characteristics can potentially benefit from IFN beta-1b treatment.

P03.069

Roquinimex (Linomide®) Treatment in Secondary Progressive and Relapsing Remitting Multiple Sclerosis: Results From 48 Week Randomized, Double-Blind, Placebo-Controlled Pilot Studies

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OBJECTIVE: To study tolerability and efficacy of roquinimex (Linomide®) given as tablets of 2.5 mg daily for 48 weeks in patients with relapsing remitting (RR) or secondary progressive (CP) form of multiple sclerosis (MS).

BACKGROUND: Roquinimex (Linomide®), a quinoline-3-carboxamide, has been shown to have an inhibitory influence in several experimental autoimmune models, including acute and chronic experimental allergic encephalitis (EAE). Two pilot studies over 24 weeks in MS patients have recently shown a decrease in disease activity when measured by monthly MRI.

DESIGN/METHODS: Thirty patients with RR MS (EDSS 1.0–4.5) and 34 patients with CP MS (EDSS 3.0–7.0) were enrolled in two separate studies. The patients were randomized to receive either roquinimex 2.5 mg daily or placebo for 48 weeks. MRI (T2 and T1-Gd-DTPA) was performed monthly during the first 24 weeks and then at week 36 and 48. The clinical status was followed at 12 week intervals using EDSS, Ambulation Index, and Disease Steps.

RESULTS: A total of 5 (out of 33) patients in the roquinimex groups and 1 (out of 31) patients in the placebo groups were withdrawn from the studies due to adverse events and/or MS related problems. In the RR study, the mean number of new enhancing lesions per visit over 48 weeks was 0.694 in the roquinimex group and 0.875 in the placebo group ($p = 0.219$). In the CP study, the mean number of new enhancing lesions was 0.406 versus 0.695 ($p = 0.786$). In the RR study, the mean change in EDSS at 48 weeks was -0.47 for the roquinimex treated group and -0.04 for the placebo group. In the CP study, the mean change in EDSS was 0.04 for the roquinimex group and +0.22 for the placebo group. Although a suggestion of positive trends were observed in both studies these differences were not statistically significant. Meta analysis of the 4 pilot studies and immunologic data will also be presented.

CONCLUSION: Although these two pilot studies did not show statistically significant differences in the parameters measured, trends compatible with the previous pilot studies were observed. Phase III studies are currently in progress in both secondary progressive (1200 patients) and RR MS (500 patients) to establish clinical efficacy of Linomide in MS.

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P03.070

Oral Cladribine for Multiple Sclerosis

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OBJECTIVE: Determine if oral cladribine (CLA) will safely lower absolute lymphocyte counts (ALC) in patients in progressive phase of multiple sclerosis (MS).

BACKGROUND: Intravenous CLA pulse therapy has been reported to be effective for treatment of the progression of phase

of MS. A therapeutic trial of subcutaneous CLA is underway. We thought oral CLA might be just as effective if the dose were adjusted.

DESIGN/METHODS: We planned a dose escalation trial of 4–6 monthly pulses to lower the ALC count below 1000/cmm with safety and tolerance. Three patients received 0.14 mg CLA for five days once every four weeks for four-six pulses. Laboratory safety tests included monthly CBCs, platelet counts, and chemistry panels. Quarterly interviews and neurologic assessments documented adverse effects, tolerance, and clinical change.

RESULTS: The ALCs were affected with the first dose chosen (nadirs: patient 1, 200; patient 2, 600; patient 3, 300/cmm). Patient 1 received five pulses; patient 2, four; patient 3, six. Treatment in patient 2 was interrupted because of a drop in total white blood cell count. ALCs below 1000 have persisted for one year except in patient 2. Adverse clinical effects which were moderately well tolerated included nausea, anorexia, mild hair loss, and pronounced aggravation of weakness and fatigue. Hemorrhatic cystitis, vaginitis, and an upper respiratory infection seemed associated with ALC nadirs. Patient 1 has subtle signs of improvement, but pts 2 and 3 have had continued progression of their MS.

CONCLUSIONS: Oral CLA will decrease ALCs for at least one year. Adverse effects are manageable. Minimal effects upon signs and symptoms suggest courses of CLA will be required.

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P03.071

Safety and Tolerability of Subcutaneous Cladribine Therapy in Chronic Progressive Multiple Sclerosis

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OBJECTIVE: To evaluate the safety and tolerability of subcutaneous (s.c.) Cladribine therapy in patients with chronic progressive multiple sclerosis (CPMS).

BACKGROUND: Cladribine, a synthetic antineoplastic agent, may favourably affect the course of CPMS. However, results of the first double blind placebo controlled trial in La Jolla showed that 2 of 29 patients developed aplastic anaemia requiring transfusions of red cells and platelets. In both cases, other possible contributory co-factors were indentified.

DESIGN/METHODS: Nineteen patients with severe (mean extended disability status score [EDSS] = 6.7) CPMS were treated on a compassionate basis with Cladribine 0.07 mg/kg/day s.c. for 5 days per cycle, repeated every 4 weeks for a total of 6 cycles. Patients underwent clinical evaluation, EDSS, and haematologic analysis at baseline, during, and after therapy for up to the next 27 months.

RESULTS: The treatment was very well tolerated with no clinically significant side effect observed. Between baseline and the end of cycle 6, mean decreases were noted in absolute lymphocyte count from 1697 to 439 (P= .001), CD4 count from 865 to 187 (P=0.00009), CD8 from 409 to 165 (P=0.05) and CD19 from 200 to 25 (P=0.00003). Platelet, granulocyte and RBC counts were unaffected. More than a year after the completion of therapy, mean CD4 and CD8 counts remained suppressed (302 and 227 respectively), but the CD19 count had recovered to 179. Significant changes in EDSS were not observed during or in the first year after therapy. Global patient evaluations of the treatment were mixed.

CONCLUSIONS: Subcutaneous Cladribine therapy, at lower doses than previously reported, was remarkably well tolerated in CPMS, with no significant myelosuppression. Profound and long-lasting suppression was noted in total lymphocyte count, CD4, CD8 and CD19 subsets.

Supported by: JANSSEN - ORTHO

P03.072

Sample Size Calculations for MRI Outcome Pilot Trials in Multiple Sclerosis: Relapsing-Remitting Versus Secondary Progressive Subgroups

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OBJECTIVE: To determine sample sizes required for MRI outcome pilot trials in relapsing-remitting (RR) and secondary progressive (SP) multiple sclerosis (MS) subgroups.

BACKGROUND: Serial brain MRI is widely used in pilot studies of new agents to monitor treatment efficacy in RR and SP MS. For pilot trials, separate sample size calculations for the SP subgroup are currently not available. The present study addresses this issue.

METHODS: The calculations are based on data from 6 months of monthly T2 weighted and gadolinium enhanced MRI in 31 RR and 28 SP untreated patients undergoing natural history studies. The calculations are for a placebo controlled, parallel groups design lasting 6 months. The sample sizes are based on boot strap analysis with an 80% likelihood of showing a given treatment effect.

RESULTS: With a single baseline scan, demonstration of a 70% reduction in newly active lesions requires 2x30 RR and 2x50 SP patients. With an extra baseline scan one month before treatment, the samples sizes are 2x20 for RR and 2x30 for SP patients.

CONCLUSION: The sample sizes required for RR patients are comparable to previous studies. Larger sample sizes are needed for the SP group, and an extra baseline scan results in a reduction in both groups. These data should be considered in planning pilot MRI outcome trials.

P03.073

Measurement of Ambulatory Function in Multiple Sclerosis

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OBJECTIVE: To characterize the relationships between continuous and ordinal measures of ambulatory function in multiple sclerosis (MS) patients.

BACKGROUND: Much of the disability caused by MS is due to impaired ambulation. The Expanded Disability Severity Scale (EDSS) and the Ambulation Index (AI) are ordinal measures of impairment based largely on the maximum distance patients can walk (Dmax) and the to time to walk 8m (T8), respectively.

DESIGN/METHODS: We prospectively determined Dmax (up to 500m), T8, EDSS, and AI in 170 ambulatory MS patients.

RESULTS: Dmax and T8 were strongly related to their ordinal counterparts (r = .86 and .91, respectively), but the continuous functional measures showed considerable variability within EDSS and AI levels that the ordinal scales did not reflect. Most of the variability occurred at EDSS 6.0 to 7.0 and AI 4 to 6, where the ordinal scales rely on the use of an ambulatory aid to characterize functional ability. The use of an aid did not predict impairment as measured by Dmax or T8; many patients who required an aid had better Dmax and T8 scores than those who did not require an aid. Dmax and T8 were also strongly related to each other, even though they provided information on different features of ambulatory function.

CONCLUSIONS: The EDSS and AI did not adequately characterize ambulatory function in MS patients, due in part to their reliance on the use of an ambulatory aid rather than a quantitative continuous measure. Dmax and T8 provide more functional information than the EDSS and AI, allowing better discrimination of differences between patients and potentially greater sensitivity to detect therapeutic effects in clinical trials.

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(54) USE OF SUBSTITUTED ADENINE DERIVATIVES FOR TREATING MULTIPLE SCLEROSIS

Verwendung von substituierten Adeninderivaten zur Behandlung von MultipleSklerose

UTILISATION DE DERIVES D'ADENINE SUBSTITUEE POUR LE TRAITEMENT DE LA SCLEROSE EN PLAQUES

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- **J. CLIN. INVEST. vol. 86, no. 5, November 1990, pages 1480 - 1488 C.J. CARRERA ET AL. 'Potent toxicity of 2-chlorodeoxyadenosine toward human monocytes in vitro and in vivo.'**
- **Science, vol. 154, 1966, p.1044-1046**
- **Adv. Exp. Med. Biol., vol. 237, 1988, p.839-42**
- **Acta Neurol. Scand., vol. 75, 1987, p.352-355**
- **J. Exp. Med., vol. 160, 1984, p.310-316**
- **Nervenarzt, vol. 66, 1995, p.299-303**

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Description

Technical Field

[0001] This invention relates to the use of substituted adenine derivatives for the manufacture of a medicament for treating multiple sclerosis.

Background of the Invention

[0002] Multiple sclerosis (MS) is the result of demyelination in the brain and spinal cord (central nervous system). Symptoms resulting from this demyelination include weakness, visual impairment, incoordination, and paresthesia (abnormal tingling). The course of the disease is largely unpredictable, but often progresses through a cycle of exacerbation of symptoms followed by remission.

[0003] Conventional treatments presently employ therapy with ACTH or corticosteroids such as prednisone. Controlled studies suggest that such treatments induce more rapid clearing of acute symptoms and signs but leave the long-term outcome of the disease unaffected. Long-term maintenance therapy with ACTH or corticosteroids is contraindicated. Evidence indicates that immunosuppressant agents have no long-term benefit. (Cecil, Textbook of Medicine, Beeson et al., eds., 15th ed., W.B. Saunders Company, Philadelphia, (1979) page 847)

[0004] The etiology of multiple sclerosis is unknown but is linked to a variety of genetic and environmental factors. Both cell-mediated and humoral immune responses, triggered by extraneous or autoantigens may contribute to the pathogenesis of multiple sclerosis. Certain immune response genes may be associated with an increased susceptibility to the disease. The disease may be mediated by T cells that recognize an as yet unidentified autoantigen. For example, experimental allergic encephalomyelitis (EAE), an animal model of demyelinating diseases such as multiple sclerosis, can be induced by immunizing mice with whole myelin or specific myelin components such as myelin basic protein.

[0005] In humans with multiple sclerosis, exacerbations are correlated with high levels of neopterin in blood and cerebrospinal fluid. Neopterin is a factor released from monocytes and macrophages in the presence of activated T-cells, thereby implicating these cells as being involved in multiple sclerosis exacerbations. (Fredrickson et al. (1987), Acta Neurol. Scand., 75:352-355; Huber et al. (1984), J. Exp. Med., 160:310-316). At the microscopic level, monocytes, microglial cells (macrophages of the central nervous system) and activated T-cells are found within the demyelinated regions of the nerve cells during multiple sclerosis exacerbations. (Cecil, Textbook of Medicine (1979), Beeson et al. (eds.), W.B. Saunders Co., Philadelphia, PA).

[0006] Various conventional treatment methodologies have been employed to ameliorate the symptoms of multiple sclerosis. Many of these are directed to use of palliative, anti-inflammatory agents. No treatment to date has had any consistent positive effect on the course of the disease.

[0007] Recently, the art has described the use of specific deoxyribosides as anti-inflammatory agents. For instance, U.S. Patent No. 4,481,197 (Rideout et al.) relates to the use of unsubstituted 3-deaza-2'-deoxyadenosine derivatives in the treatment of inflammation. U.S. Patent No. 4,381,344 (Rideout et al.) relates to a process for the synthesis of deoxyribosides that utilizes a bacterial phosphorylase.

[0008] A deoxyriboside derivative, 2-chloro-2'-deoxyadenosine (CdA), has been found to be an effective agent for the treatment of chronic lymphocytic leukemia and some T cell malignancies. (Carson et al. (1984) Proc. Natl. Acad. Sci. U.S.A., 81:2232-2236; Piro et al. (1988), Blood 72:1069-1073) The pharmacokinetics of orally and subcutaneously administered 2-chloro-2'-deoxyadenosine in the treatment of chronic lymphocytic leukemia have been described and compared. (Liliemark et al. (1992) Journal of Clinical Oncology, 10, (10): 1514-1518; Juliusson et al. (1992) Blood, 80 (Suppl. 1): 1427) Chronic lymphocytic leukemia is a malignancy of B lymphocytes that bear the Leu-I surface antigen.

[0009] The Leu-I B cells represent a minor proportion of the normal pool of B lymphocytes, usually less than 20 percent. The Leu-I B cells express surface markers that are typically found on monocytes (Mac-I antigen) and T-lymphocytes (Leu-I antigen). Approximately 10 percent of patients with chronic lymphocytic leukemia exhibit accompanying autoimmunity, and recently, Leu-I B cells have been implicated in the pathogenesis of autoimmune diseases.

[0010] Phase I clinical trials on human patients with chronic lymphocytic leukemia indicate that infusion of increasing doses of 2-chloro-2'-deoxyadenosine [0.1-0.5 milligrams per kilogram of body weight per day (mg/kg/day)] yielded increasing plasma concentrations of the drug [10-50 nanomolar (nM)]. Those infusions indicated that the drug was well tolerated and did not induce nausea, vomiting or fever. The dose-limiting toxicity was bone marrow suppression, which usually occurred at doses greater than about 0.2 mg/kg/day or at plasma levels of greater than about 20 nM.

[0011] Other studies, Montgomery et al. (1959) J. Am. Chem. Soc., 82:463-468, indicated that 2-fluoroadenosine exhibits a relatively high degree of cytotoxicity. Those workers reported that C57 black mice implanted with Adenocarcinoma 755 (Ad755) could tolerate only about 1 milligram per kilogram of body weight. 2-Fluoroadenosine was found to be inactive at that level against Ad755 as well as leukemia L1210 and the Erlich ascites tumor.

[0012] U.S. Patent No. 4,751,221 and its division No. 4,918,179 to Watanabe et al. describe the synthesis and use

of several 2-substituted-2'-deoxy-2'-fluoroarabino-furanosyl nucleosides including adenine derivatives. Those compounds were said to have anti-tumor and antitrypanosomal biological activities. Cytotoxicity data showing anti-tumor activity of 2-amino-6-thiopurine, guanine and thiopurine derivatives against murine and human cell lines were reported.

[0013] U.S. Patent No. 5,034,518 to Montgomery et al. teaches the synthesis of 2-substituted-2'-deoxy-2'-fluoroaraadenosines. Those compounds were said to have anticancer activity, and data for prolongation of life of mice transplanted with P388 leukemia cells were provided.

[0014] The biochemical activity of 2-CdA in cells has been reviewed by Ernest Beutler. (The Lancet (1992), 340: 952-956 - incorporated herein by reference)

[0015] The 2',3'-dideoxynucleosides are phosphorylated at the 5'-position in T cells to form the 5'-nucleotide triphosphate derivatives. Those derivatives are well known to be substrates for reverse transcriptase molecules. (Ono et al. (1986) Biochem. Biophys. Res. Comm., 2:498-507)

[0016] Those 2',3'-dideoxynucleoside 5'-triphosphates are also utilized by mammalian DNA polymerases beta and gamma. (Waquar et al. (1984) J. Cell. Physiol., 121:402-408) They are, however, poor substrates for DNA polymerase-alpha, the main enzyme responsible for both repair and replicative DNA synthesis in human lymphocytes. In part, these properties may explain the selective anti-HIV activity of the 2',3'-dideoxynucleosides.

[0017] Chan et al. (1982) J. Cell Physiol., 111:28-32 studied the pathways of pyrimidine nucleotide metabolism in murine peritoneal macrophages and monocytes, and reported undetectable levels of deoxycytidine kinase or thymidine kinase in these cells. High levels of adenosine kinase were found, however.

[0018] Similar high levels of adenosine kinase have been found in human monocytes and human monocyte-derived macrophages (MDM). MDM were found to exhibit about one-tenth to about one-fourth the nucleoside kinase activity of GEM T lymphoblasts (e.g. ATCC CCL 119) toward uridine, deoxycytidine and thymidine, and about two-thirds the adenosine kinase activity of GEM cells. In addition, that adenosine kinase activity of MDM cells was at least about 10-fold higher than any of the other kinase activities. Those studies also indicated relatively low levels of nucleoside phosphorylation using AZT, dideoxycytidine (ddC) and 2',3'-dideoxyadenosine (ddA) in intact GEM T lymphoblasts and still lower levels with the MDM.

[0019] Several 2-substituted adenosine derivatives have been reported not to be deaminated by adenosine deaminase. For example, Coddington (1965) Biochim. Biophys. Acta, 99:442-451 reported that deoxyadenosine-1-N-oxide, as well as 2-hydroxy-, 2-methyl-, 2-chloro-, 2-acetamido-, and 2-methylthio-adenosines were neither substrates nor inhibitors for adenosine deaminase. Montgomery, in Nucleosides, Nucleotides, and Their Biological Applications, Rideout et al. eds., Academic Press, New York, page 19 (1983) provides a table of comparative K_m and V_{max} data for the deamination of adenosine, 2-halo-adenosines 2-halo-deoxyadenosines and 2-fluoro-arabinoadenosine that also indicates that those 2-halo adenine derivatives are poor substrates for the enzyme relative to adenine itself. Stoeckler et al. (1982) Biochem. Pharm., 31:1723-1728 reported that the 2'-deoxy-2'-azidoribosyl and 2'-deoxy-2'-azidoarabinosyl-adenine derivatives were substrates for human erythrocytic adenosine deaminase, whereas work of others indicated 2-fluoroadenosine to have negligible activity with adenosine deaminase.

[0020] 2-Chloro-2'-deoxyadenosine is phosphorylated by non-dividing (normal) human peripheral blood lymphocytes and is converted to the 5'-triphosphate. This adenine derivative is not catabolized significantly by intact human cells or cell extracts, and is phosphorylated efficiently by T lymphocytes. (Carson et al. (1980) Proc. Natl. Acad. Sci. USA, 77:6865-6869)

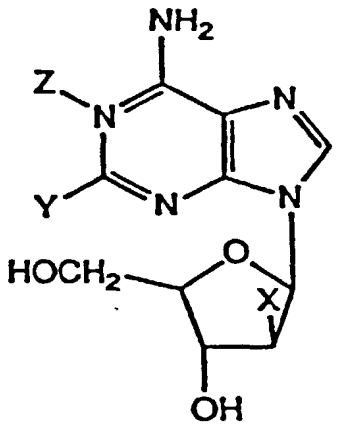
[0021] As discussed before, high levels of adenosine kinase have been found in murine peritoneal macrophages and in human monocytes. Adenosine kinase can phosphorylate 2'-deoxyadenosine derivatives, but does so less efficiently than deoxycytidine kinase. (Hershfield et al. (1982) J. Biol. Chem., 257:6380-6386)

[0022] Chemotherapeutic agents are described hereinafter that may be employed as therapeutic agents in the treatment of multiple sclerosis.

Summary of the Invention

[0023] The present invention relates the use of an substituted derivative for the preparation of a medicament for treating multiple sclerosis. The medicament has a pharmacologically acceptable carrier and a substituted adenine derivative dissolved or dispersed therein. The substituted adenine derivative is present in the pharmacologically acceptable carrier in an amount sufficient to provide a therapeutically effective dose over the course of treatment.

[0024] The substituted adenine derivatives useful for treating multiple sclerosis nay be represented by Formula I having a structural formula corresponding to:



wherein Z is O^- or absent,
Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, provides a soluble adenine derivative and whose presence on the adenine moiety inhibits deamination of the adenine derivative by adenosine deaminase; and
X is hydrogen or fluoro, with the proviso that when Z is absent, Y is not hydrogen.

[0025] Particularly preferred compounds of Formula I are free of the Z group; i.e, Z is absent, and contain a halo group at the 2-position. The most preferred compounds are 2-chloro-2'-deoxyadenosine and 2-chloro-2'-deoxy-2'-arafluoroadenosine.

[0026] Methods for synthesizing all of the above compounds are indicated in U.S. Patent 5,106,837 (Carson et al., April 21, 1992, incorporated herein by reference).

[0027] The invention teaches that the disease condition of a patient having multiple sclerosis may be ameliorated by administration of an amount of the above-described composition having a sufficient quantity of the compound of Formula I to provide a therapeutically effective dose. Exemplary dosages range from about 0.04 to about 1.0 mg/kg/day, with dosages of about 0.04 to about 0.2 mg/kg/day being more preferred. Typically, the amount is sufficient to provide a concentration in the patient's plasma of about 0.5 nanomolar (nM) to about 50 nM, more preferably of about 1 nM to about 10 nM.

[0028] Preferably, the agent contemplated for use in the present invention is a 2-halo-2'-deoxyadenosine (2-halo-2'-deoxy-9,1'-beta-ribofuranosyladenine) or a 2-halo-2'-deoxy-2'-arafluoroadenosine, and most preferably the halo group is chloro.

[0029] A further aspect contemplated by the present invention comprises the use of subcutaneous injection for administering an effective amount of the active ingredient (agent) of the invention for treating multiple sclerosis.

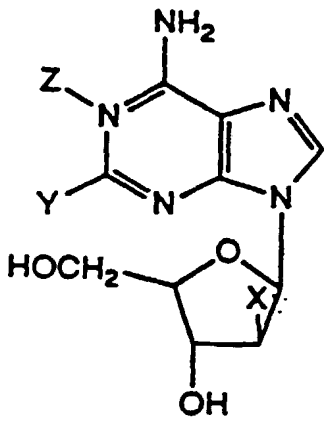
[0030] An alternative aspect contemplated by the present invention comprises the peroral administration of an effective amount of the active ingredient (agent) of the invention in a method of treating disease. Preferred compounds of Formula I for oral administration include compounds in which X is fluoro.

[0031] In each of the before-described methods, the substituted 2'-deoxyadenosine derivative is administered in a therapeutically effective amount. The effect of a compound of Formula I is dependent upon the route of administration and upon the time and dosage. As a consequence, one can tailor the dosage and duration for which a particular compound is administered to the stage of the disease and the condition of the patient being treated. Where the stage of multiple sclerosis is advanced or life-threatening, treatment may be more aggressive, and a therapeutically effective amount is an amount that is sufficient to kill at least 50 percent of the monocytes present but is less than that which substantially impairs bone marrow function as determined by usual procedures when administration is in vivo. The monocyte killing amount of a compound of Formula I is another measure of a therapeutically effective dose and monocyte death is measured at a time seven days after the initial administration.

Detailed Description of the Invention

A. Compounds

[0032] The present invention contemplates the use of substituted adenine derivatives, i.e. substituted-2'-deoxy-arabinofuranosyladenine, for treating multiple sclerosis. Preferred substituted adenine derivatives have a structure represented by the following formula, viz. Formula I:



wherein Z is an oxide radical (O^-) or is absent;
Y is hydrogen or a radical containing one to about twenty atoms that is free from net ionic charge at physiological pH values, provides a soluble adenine derivative, and whose presence on the adenine moiety inhibits deamination of the adenine derivative by adenosine deaminase; and
X is hydrogen or fluorine, with the proviso that Y is hydrogen only when Z is present.

[0033] Preferably, Y is chloro. Other Y substituents may be selected from the group consisting of lower alkyl, lower alkanoylamido, lower alkylthio and hydroxyl radicals. In particularly preferred embodiments, when Y is chloro, X is fluorine.

[0034] The preferred compound included in Formula I is 2-chloro-9,1'-beta-D-2'-deoxyribosyladenine, otherwise known as 2-chlorodeoxyadenosine or CdA.

[0035] Of the compounds of Formula I, those where X is fluoro are among the preferred compounds for use by oral administration.

[0036] Other illustrative compounds included in Formula I are:

2-bromo-9,1'-beta-D-2'-deoxyribosyladenine;
2-methyl-9,1'-beta-D-2'-deoxyribosyladenine;
2-fluoro-9,1'-beta-D-2'-deoxyribosyladenine;
2-acetoamido-9,1'-beta-D-2'-deoxyribosyladenine;
2-methylthio-9,1'-beta-D-2'-deoxyribosyladenine;
2-chloro-9,1'beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
2-bromo-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
2-(N-acetamido)-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine;
2-methylthio-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine.

[0037] Further illustrative of compounds of Formula I include the following arabinofuranosyl derivatives of adenine:

2-methyl-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
2-isopropyl-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
2-hydroxy-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
2-chloro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-l-N-oxide;
2-fluoro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
2-bromo-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
2-methyl-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
2-(N-acetamido)-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
2-hydroxy-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-l-N-oxide;
2-(2-methylbutyl)-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-l-N-oxide;
2-fluoro-9,1'-beta-D-2'-deoxyadenosine-l-oxide;
and
2-chloro-9,1'-beta-D-2'-deoxyadenosine-1-oxide.

[0038] It is noted that when X is hydrogen the sugar ring can be named as a 2'-deoxyribosyl or 2'-deoxyarabinofuranosyl radical. Both nomenclatures are utilized herein. When the class of compounds embraced by Formula I is discussed, all of the compounds are considered herein as derivatives of arabinose. However, when specific compounds of the subclass where X = H are discussed, the more familiar deoxyribose nomenclature is used, such as in deoxyadenosine. These compounds are also referred to herein more simply as adenine derivatives.

[0039] In the above formulas, and in all other formulas shown herein, hydrogen atoms on the purine and furanosidyl rings that are not needed to show conformation about a particular bond are not shown. Thus, the 8-position adenine hydrogen is not shown.

[0040] It is also to be understood that the D isomers of compounds of the formulas are the isomers contemplated. It is further to be noted that the designation "halo" used herein is meant to include fluorine, chlorine and bromine derivatives, and to exclude iodine derivatives, which are unstable and decompose, and astatine derivatives that are radioactive. Where specific halogen derivatives are intended, those compounds are named specifically.

[0041] As used herein, "a substituent free from net ionic charge" includes both charged and uncharged radicals, wherein when the substituent radical is charged, an internal zwitterionic charge pair is present that results in the absence of a net ionic charge for the molecule at physiologic pH values. N-oxide compounds are exemplary of such substituents.

[0042] As used herein, a "soluble adenine derivative" is an adenine derivative which is able to dissolve and remain soluble in a body fluid such as blood at a therapeutically effective dose as is discussed hereinafter.

[0043] As used herein, a "substituent whose presence on the adenine moiety inhibits deamination of an adenine derivative by adenosine deaminase" is one that, when 100 microliters of a 1 millimolar solution of the substituted adenine derivative is incubated for three hours at room temperature with 25 units of calf spleen adenosine deaminase (1 unit catalyzes the deamination of 1 micromole of adenosine per minute), produces a single UV-absorbing spot upon cellulose-thin layer chromatography of the reaction mixture whose R_f value is the same as that of the substituted adenine derivative used.

[0044] The metabolism of a compound by adenosine deaminase can be investigated by the following procedure. The individual nucleosides, at concentrations from 5-200 μM in 10 mM sodium phosphate, pH 7.5, are incubated at 18-20 degrees C with 0.01 EU/ml calf intestinal adenosine deaminase. The change in the optical density at 265 nm and 250 nm is monitored spectrophotometrically. The K_m and V_{max} values are determined by the Lineweaver-Burke method, utilizing the ΔE^{M}_{265} between adenosine and inosine.

[0045] The ratio V_{max}/K_m also provides a measure of relative efficiency of deamination by the enzyme. A substituent that provides a V_{max}/K_m ratio that is about 1 percent or less than that for the ratio obtained using 2'-deoxyadenosine is also a "substituent whose presence on the adenine moiety inhibits deamination of an adenine derivative by adenosine deaminase."

[0046] As used herein, lower alkyl radicals include C_1-C_6 straight chain, branched and cyclic alkyl groups, for example, methyl, ethyl, n-butyl, t-butyl, n-hexyl, 1-ethylbutyl, cyclopentyl, cyclohexyl and the like. Lower alkanoylamido radicals include C_1-C_6 radicals, for example, formamido, acetylamido, propionamido, hexamoylamido and the like. Lower alkylthio radicals include C_1-C_6 straight chain, branched and cyclic alkyl groups as discussed above linked to a thio radical.

[0047] The pharmacologically acceptable salts of a compound of the above Formula are also utilized. The phrase "pharmacologically acceptable salts," as used herein, refers to non-toxic acid addition salts that are generally prepared by reacting a compound with a suitable organic or inorganic acid. Representative salts include the hydrochloride, hydrobromide, sulfate, phosphate, citrate, acetate, maleate and the like.

B. Compositions

[0048] A compound of Formula I dissolved or dispersed in or together with a pharmacologically acceptable carrier constitutes a composition of this invention.

[0049] A compound of Formula I and its pharmacologically acceptable salts are useful in both short and long term treatment. For instance, a 2-substituted-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine is administered to the patient internally, e.g., subcutaneously by injection, parenterally, orally, or rectally as a suppository, in an effective amount.

[0050] Although a compound of Formula I and its pharmacologically acceptable salts can be administered as the pure chemical, it is preferred that it be administered as a pharmaceutical composition. In either event, it is administered in an amount sufficient to provide a therapeutically effective dose as is discussed hereinafter.

[0051] Accordingly, the present invention utilizes a pharmaceutical composition comprising a therapeutically effective dose of a compound of Formula I or a pharmacologically acceptable salt thereof, hereinafter referred to as the "active ingredient" or "agent," dissolved or dispersed in a pharmacologically acceptable carrier or diluent.

[0052] A pharmaceutical composition is prepared by any of the methods well known in the art of pharmacy all of which involve bringing into association the active compound and the carrier therefor. For therapeutic use, a compound utilized in the present invention can be administered in the form of conventional pharmaceutical compositions. Such compositions can be formulated so as to be suitable for oral, subcutaneous, or parenteral administration, or as suppositories. In these compositions, the agent is typically dissolved or dispersed in a physiologically tolerable carrier.

[0053] A carrier or diluent is a material useful for administering the active compound and must be "pharmacologically acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. Thus, as used herein, the phrases "physiologically tolerable" and "pharmacologically acceptable" are used interchangeably and refer to molecular entities and compositions that do not produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a mammal. The physiologically tolerable carrier can take a wide variety of forms depending upon the preparation desired for administration and the intended route of administration.

[0054] As an example of a useful composition, a compound of Formula I can be utilized in liquid compositions such as sterile suspensions or solutions, or as isotonic preparations containing suitable preservatives. Particularly well-suited for the present purposes are injectable media constituted by aqueous injectable isotonic and sterile saline or glucose solutions. Additional liquid forms in which these compounds can be incorporated for administration include flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, peanut oil, and the like, as well as elixirs and similar pharmaceutical vehicles.

[0055] The agents can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain stabilizers, preservatives, excipients, and the like in addition to the agent. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic.

[0056] Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq.

[0057] An agent of Formula I can also be used in compositions such as tablets or pills, preferably containing a unit dose of the compound. To this end, the agent (active ingredient) is mixed with conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate, gums, or similar materials as non-toxic, physiologically tolerable carriers. The tablets or pills can be laminated or otherwise compounded to provide unit dosage forms affording prolonged or delayed action.

[0058] It should be understood that in addition to the aforementioned carrier ingredients the pharmaceutical formulation described herein can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

[0059] The tablets or pills can also be provided with an enteric layer in the form of an envelope that serves to resist disintegration in the stomach and permits the active ingredient to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, including polymeric acids or mixtures of such acids with such materials as shellac, shellac and cetyl alcohol, cellulose acetate phthalate, and the like. A particularly suitable enteric coating comprises a styrene-maleic acid copolymer together with known materials that contribute to the enteric properties of the coating. Methods for producing enteric coated tablets are described in U.S. Patent 4,079,125 to Sipos, which is herein incorporated by reference.

[0060] The term "unit dose", as used herein, refers to physically discrete units suitable as unitary dosages for

administration to patients, each such unit containing a predetermined quantity of the agent calculated to produce the desired therapeutic effect in association with the pharmaceutically acceptable diluent. Examples of suitable unit dosage forms in accord with this invention are tablets, capsules, pills, powder packets, granules, wafers, cachets, teaspoonfuls, dropperfuls, ampules, vials, segregated multiples of any of the foregoing, and the like.

[0061] Administration of the compound by subcutaneous injection is a particularly attractive mode of administration due to the favorable pharmacokinetics of this mode of administration.

[0062] Oral administration of the compound is also an attractive mode of administration. One drawback usually associated with oral administrations of bioactive nucleoside compounds, however, is their potential decomposition in the acidic conditions of the stomach. That is, the glycosidic bond tends to hydrolyze under acid conditions.

[0063] However, where oral administration is desired, substitutions on the 2-position of the adenine ring of the compound of Formula I are utilized along with a 2'-fluoro-substituted arabinofuranosidyl ring.

[0064] Marquez et al. (1987) Biochem. Pharm., 36:2719-2722 reported preparation of 2'-fluoro-2',3'-dideoxyribose and 2'-fluoro-2',3'-dideoxyarabinose derivatives of adenine. Their findings stated that both derivatives were stable at a pH value of 1 at 37 degrees C, whereas dideoxyadenosine had a half-time of 35 seconds under those conditions.

[0065] The ability of an adenine derivative to be or not to be a substrate for adenosine deaminase is more a function of the 2-substituent or lack thereof on the adenine portion of the molecule than a function of substituents on the linked sugar ring portion, at least as far as the substituents on both rings herein are concerned.

C. Methods

[0066] As noted earlier, a method of treating multiple sclerosis is contemplated herein. Broadly in that method, a patient having multiple sclerosis is treated with a composition containing a pharmacologically acceptable carrier having dissolved or dispersed therein, as an active ingredient, a substituted adenine derivative (substituted 2'-deoxy-adenosine) whose structure corresponds to that of previously discussed Formula I. The substituted adenine derivative is present in the composition in an amount sufficient to provide a therapeutically effective dose over the period of contacting. The above treatment is typically repeated periodically such as weekly or monthly over a time period of several months to about one year.

[0067] The amount of a compound of Formula I present in a composition and used in a method as described above is a function of several variables. Among those variables is the method of administration. Exemplary concentrations for various modes of administration are illustrated hereinafter.

[0068] When the administration is in vivo, the amount administered is less than that which substantially impairs bone marrow functions as determined by usual procedures. An amount sufficient to kill at least about 50 percent of the monocytes originally present while not substantially impairing bone marrow function over the course of the administration of the agent is one way of defining a therapeutic dose.

[0069] The above amount of a 2'-deoxyadenine derivative of Formula I or its pharmacologically acceptable salt present in the composition is also an amount sufficient to provide about 0.04 to about 1.0 mg/kg of body weight of the treated host mammal per day, more preferably about 0.04 to about 0.20 mg/kg/day, more preferably still at about 0.05 to about 0.15 mg/kg/day and most preferably about 0.1 mg/kg/day, when given in vivo. This amount is another way of defining a therapeutically effective dose that is particularly useful when a compound of Formula I is administered by infusion.

[0070] The molar plasma concentration of the compound of Formula I or the pharmacologically acceptable salts thereof during treatment is preferably in the range of about 1 nanomolar (nM) to about 100 nM, particularly about 5 nM to about 50 nM, and more preferably about 10 nM to about 20 nM. Molarity of the 2'-deoxyadenine derivative in plasma of the treated (administered to) patient thus provides still another measure of a therapeutically effective dose from which the amount in a composition can be calculated.

[0071] It is to be understood that the above therapeutically effective dosages need not be the result of a single administration, and are usually the result of the administration of a plurality of unit doses. Those unit doses can in turn comprise portions of a daily or weekly dosage, and thus, the therapeutically effective dose is determined over the period of treatment (contacting).

[0072] Oral administration and subcutaneous injection are preferred modes of administration, as already noted. To achieve the desired plasma concentration of the agent, a range of doses can be employed depending upon the specific mode of administration, objective of the particular treatment, the particular compound being used, and like considerations.

[0073] For example, for oral administration, the daily dose can be about 0.04 to about 1.0 mg/kg of body weight, more preferably about 0.04 to about 0.20 mg/kg/day, more preferably still at about 0.05 to about 0.15 mg/kg/day, and most preferably about 0.1 mg/kg body weight. In general, the amount of active substituted adenine derivative administered can vary over a relatively wide range to achieve, and preferably maintain, the desired plasma concentration.

[0074] Unit dosage forms of the adenine derivative can contain about 0.1 milligrams to about 15 milligrams thereof.

A preferred unit dosage form contains about 0.1 to about 1 milligram of agent and can be administered 2 to 5 times per day. However, it should be noted that continuous infusion at a rate designed to maintain the above described plasma concentration is also contemplated.

[0075] Duration of a particular treatment can also vary, depending on severity of the disease, whether the treatment is intended for an acute manifestation or for prophylactic purposes, and like considerations. Typical administration lasts for a time period of about 5 to about 14 days, with a 7-day time course being usual. Courses (cycles) of administration can also be repeated at monthly intervals, or parenteral unit dosages can be delivered at weekly intervals. Oral unit dosages can be administered at intervals of one to several days to provide the determined therapeutically effective dose. Thus, in vivo administration of a before-discussed dosage over a time period of about 5 to about 14 days or at weekly or daily intervals provides an amount sufficient to kill at least about 50 percent of the originally present monocytes.

[0076] This method of treatment produces a decrease in the level of monocytes in the blood due to the toxicity of the utilized compounds of Formula I toward monocytes. This method can be used to reduce the number of monocytes circulating in a treated mammal's blood stream by about 90 percent of the number present prior to treatment over a seven day treatment period with the level of circulating monocytes returning to pretreatment levels about two weeks after the treatment stopped. This exemplary study is illustrated hereinafter.

[0077] A less aggressive treatment regimen is also therefore contemplated. Here, a before-described dosage, e.g., plasma concentration, is again utilized, but for a shorter contact time course so that monocyte function is impaired, but the monocytes are not substantially killed as is the result of the before- discussed treatment regimen. Impairment of monocyte function is herein defined as a reduction of at least about 25 percent in the spontaneous secretion of interleukin-6 (IL-6) by monocytes cultured in the presence of a compound of Formula I for a time period of 72 hours. A useful assay for monocyte impairment is discussed hereinafter.

[0078] In an exemplary treatment regimen, a compound of Formula I is administered in an amount of about 0.04 to about 1.0 mg/kg/day, more preferably about 0.04 to 0.20 mg/kg/day, more preferably still about 0.05 to about 0.15 mg/kg/day, and most preferably about 0.1 mg/kg/day. Such treatments typically provide a plasma concentration of about 0.5 nM to about 50 μM, and more preferably about 10 nM to about 10 μM. That single administration is repeated periodically such as weekly over a time period of several months, e.g. about three to about nine months. In usual practice, treatments are administered over a period of about five to seven days and are repeated at about three to about four week intervals for several months, e.g. about three to about nine months.

[0079] Such an administration can be carried out on an out-patient basis for humans using an intravenous infusion lasting about 2 to about 4 hours in a doctor's office. As such, the treatment is far less invasive than is a continuous infusion over a period of several days that usually requires a hospital stay for the host mammal; i.e., human patient. A less invasive continuous infusion method that employs a pump linked to a catheter that automatically infuses a predetermined dosage permits the patient to be ambulatory during the infusion.

[0080] Any of the before-discussed methods can be carried out while the patient is continuing therapy with a previous drug or drugs, or after cessation of such prior treatment. When a patient is removed from a prior even partially effective treatment, a flare-up (exacerbation) of symptoms sometimes occurs that typically abates after several months. In addition, where a prior treatment regimen is halted while an above method is practiced, that prior treatment can be continued after cessation of an above method, often with quite positive results.

[0081] Dosage schedules and protocols for administering 2-chlorodeoxyadenosine to treat patients having disease conditions other than multiple sclerosis have been reviewed in the literature. (Ernest Beutler (1992), The Lancet, 340: 952-956) To a first approximation, the pharmacokinetics of 2-chlorodeoxyadenosine and its effect upon monocyte levels are independent of the disease condition being treated.

D. Compound Synthesis

[0082] A compound useful herein where Z is absent can be prepared by condensing an appropriately substituted adenine directly with an appropriately substituted sugar ring as by the techniques described in Montgomery et al., (1986) J. Med. Chem., 29:2389-2392, by the method taught in U.S. Patent No. 4,082,911, or as described in the citations of Herdewijn at al. (1987) J. Med. Chem., 30:2131-2137, which disclosures are incorporated herein by reference. An appropriately substituted adenine can be prepared by following reported literature syntheses or analogous syntheses. Still further, Wright et al. (1987) J. Org. Chem., 35:4617-4618 recently prepared 2-chloro- and 2-bromo-2'-deoxyadenosines by direct reaction of the appropriate 2,6-dihalo purine with a 3',5'-protected-alpha-l-chlororibose using sodium hydride in acetonitrile, followed by treatment with methanolic ammonia at 60 degrees C to deprotect the resulting 3',5'-hydroxyls and form the 6-amino group of the finally produced adenosine. Fukukawa et al. (1983) Chem. Pharm. Bull., 31(5):1582-1592 also report syntheses of 2'-deoxy-2'-arahalo-substituted adenosine derivatives.

[0083] The 2'-deoxy-2'-fluoroarabinofuranosyladenine compounds of the present invention are produced as described hereinafter in the Examples. The synthesis is similar to that taught in Marquez at al. (1987) Biochem. Pharmacol., 36:2719-2722, herein incorporated by reference, in which 6-chloropurine is condensed with 3-O-acetyl-5-O-

benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide. The functionalized halosugar is produced according to the method reported by Reichnan et al. (1975) J. Carbohyd. Res., 42:233 and the 2'-deoxy-2'-fluoro-arabinofuranosyladenine compound is obtained by ammonolysis with concentrated methanolic ammonia which removes the protective groups. Syntheses of 2-substituted-2'-deoxy-2'-arafluoroadenosines are also described in U.S. Patents No. 4,918,179 and No. 5,034,518, whose disclosures are incorporated by reference.

[0084] The adenosine-1-N-oxide group of compounds, i.e, where Z is present, is of particular interest since those materials, per se, are most likely not incorporated into a growing polynucleotide chain because the presence of the N-oxide group probably interferes with hydrogen bonding during that synthesis. Rather, it is believed that the N-oxide compounds are reduced by an endogenous reductase prior to their incorporation into and termination of the growing chain.

[0085] Nevertheless, being free from a net ionic charge, but possessing an internal zwitterionic charge pair, the N-oxide compounds can penetrate cell membranes. Those compounds are also somewhat more water-soluble than are the corresponding un-oxidized compounds.

[0086] Without wishing to be bound by theory, it is nevertheless believed that the N-oxide compounds enter the cell and are phosphorylated, in keeping with the report of such phosphorylation in Lindberg et al. (1967) J. Biol. Chem., 242:350-356. A pool of such derivatives is maintained intracellularly until such time as the N-oxide function is reduced and the nucleotide is incorporated to terminate the appropriate, growing polynucleotide chain.

[0087] The 1-N-oxide compounds are readily prepared by the method of Klenow et al. (1961) Biochim. Biophys. Acta, 52:386-389, with slight modification, as discussed hereinafter.

[0088] The present invention is further illustrated by the following examples which are not intended to limit the scope of the invention in any way.

Example 1

Treatment of Multiple Sclerosis with CdA

[0089] A study of four patients with chronic multiple sclerosis was undertaken. Each patient was first examined for normal hepatic, renal, and bone marrow functioning to establish baseline values. Each of the patients was then treated with CdA dissolved in sterile preservative-free isotonic saline. The CdA was administered intravenously at a dosage of 0.1 mg/kg each day for a total of seven days. Each patient received six courses of intravenous therapy, once monthly for a total of six months. Patients were examined on a daily basis while hospitalized. During that time, daily blood counts and twice weekly blood chemistries were performed on each patient. CdA levels were also measured in blood and spinal fluid.

[0090] The neurologic function of each of these patients was measured using the expanded Krutzke disability status scale (EDSS), and the Scripps neurologic rating scale (SNRS).

[0091] There was no evidence of any significant toxic side effects. None of the four patients exhibited any nausea, vomiting, skin rash, or hepatic or renal dysfunction. Each of the patients developed lymphopenia (reduction in the level of lymphocytes in the blood), with absolute lymphocyte counts being suppressed 0.5 to about 10 percent for more than one year.

[0092] Monocyte levels dropped after each treatment. For example, in one patient, monocytes dropped 40 percent after the first treatment, and were substantially absent after each of the remaining five treatments. For another patient, monocytes were substantially absent after two treatments, and depleted by about 85, 50, 40 and 73 percents after the other four treatments.

[0093] In some cases, there was leukopenia (reduction in the level of total white blood cells). There was also a modest macrocytosis in all patients lasting for six to eight months after cessation of treatment. However, the platelet counts of all four patients remained within the normal range. In essence, there was no evidence of toxicity in these four patients with normal marrow, hepatic and renal functions. Likewise, the side effects of CdA were imperceptible in these four patients.

[0094] Measurement of neurologic function using the EDSS and SNRS scales provided evidence of improvement in all four patients during treatment with CdA. Cerebrospinal fluid studies (CSF) showed a marked drop in lymphocyte counts and, quite remarkably, complete disappearance of IgG oligoclonal bonds in all cases. There was no significant change in total CFS IgG.

[0095] In particular, the SNRS data demonstrated between 5 and 50 percent improvement from baseline pre-treatment values in all patients. One of the four patients was completely bed-ridden at the beginning of the treatment, and this patient was able to walk with the aid of a walker by the end of the treatment. All patients reported subjective feelings of improved energy and stamina.

Example 2

Treatment of Multiple Sclerosis with CdA

[0096] The study indicated above in Example 1 involving four patients was then enlarged. A double-blind placebo study involving 50 patients was performed to further demonstrate the effectiveness of 2-CdA for treating multiple sclerosis. The dosage schedules and protocols for this second study were similar or substantially the same as the dosage schedules and protocols employed in Example 1. The same two neurologic rating scales were employed, i.e. the SNRS scale and the EDSS scale. 28 patients were tested with the SNRS scale; 23 patients were tested with the EDSS scale. The SNRS scale is substantially more sensitive than the EDSS scale. The inventor's most recent data indicate that a highly significant improvement (p=0.0004) was observed in patients treated with 2-CdA as compared with placebo in the 28 patients tested for changes in the SNRS scale.

Table I

Changes in SNRS

	Absolute	Relative	
CdA	4.83 ± 5.71	0.076 ± 0.089	(N=14)
Placebo	-4.40 ± 5.14	-0.062 ± 0.071	(N=14)
	p=0.0004	p=0.0005	

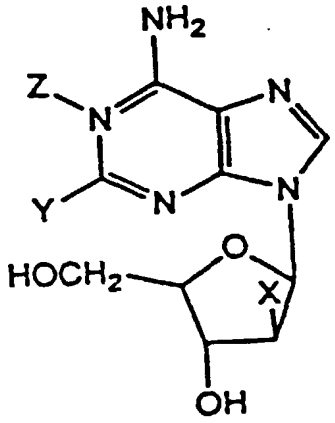
Changes in EDSS

	Absolute	Relative	
CdA	-0.018 ± 0.222	-0.011 ± 0.081	(N=12)
Placebo	0.038 ± 0.9233	0.039 ± 0.240	(N=11)
	p=0.84	p=0.50	

SNRS	0	100
EDSS	10	1
	Worst	Best

Claims

1. Use of a substituted adenine derivative having a structure represented by the formula:



or a pharmacologically acceptable salt thereof
where:

Z is O^- or absent;
Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, produces a soluble adenine derivative, and whose presence on the adenine moiety inhibits deamination of said adenine derivative by adenosine deaminase; and
X is hydrogen or fluoro,
with the proviso that when Z is absent, Y is not hydrogen for the preparation of a medicament for treating multiple sclerosis.

2. The use of claim 1, wherein Z is absent and Y is halo group.

3. The use of claim 1 wherein said substituted adenine is 2-chloro-2'-deoxyadenosine.

4. The use of any one of claims 1 to 3, wherein the treatment is by application of the adenine derivative to the patient's serum.

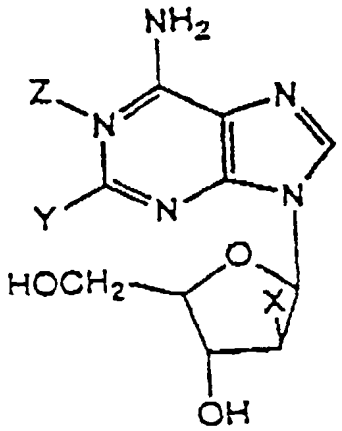
5. The use of any one of claims 1 to 4, wherein the medicament is in the form of a subcutaneous injectable medium.

6. The use of any one of claims 1 to 4, wherein the medicament is in the form of an intravenous infusion.

7. The use of any one of claims 1 to 4, wherein the medicament is in the form of capsules, powders or granules, in the form of tablets or pills that are optionally provided with an enteric layer, in the form of a suppository, in the form of a solution, suspension or elixir or in the form of liposomes.

Patentansprüche

1. Verwendung eines substituierten Adeninderivats mit einer Struktur der Formel:



oder eines pharmakologisch annehmbaren Salzes davon,
worin:

Z O^- ist oder nicht vorliegt;

Y Wasserstoff oder ein Substituent ist, welcher 1 bis etwa 20 Atome aufweist, der frei ist von einer Netto-Ionenladung bei physiologischen pH-Werten, ein lösliches Adeninderivat erzeugt und dessen Anwesenheit auf dem Adeninrest eine Desaminierung des Adeninderivats durch Adenosindesaminase inhibiert; und

X Wasserstoff oder Fluor ist,

mit der Maßgabe, daß, wenn Z nicht vorliegt, Y nicht Wasserstoff ist, für die Herstellung eines Medikamentes zur Behandlung von Multiple Sklerose.

2. Verwendung gemäß Anspruch 1, worin Z nicht vorliegt und Y eine Halogengruppe ist.

3. Verwendung gemäß Anspruch 1, worin das substituierte Adenin 2-Chlor-2'-desoxyadenosin ist.

4. Verwendung gemäß einem der Ansprüche 1 bis 3, worin die Behandlung durch die Anwendung des Adeninderivats auf das Serum des Patienten geschieht.

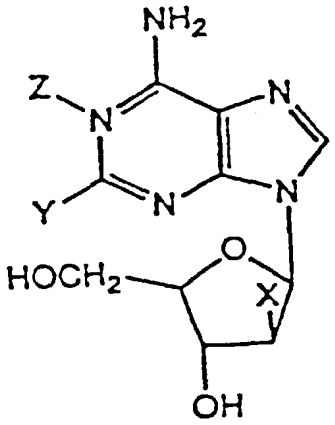
5. Verwendung gemäß einem der Ansprüche 1 bis 4, wobei das Medikament in Form eines subkutanen injizierbaren Mediums vorliegt.

6. Verwendung gemäß einem der Ansprüche 1 bis 4, wobei das Medikament in Form einer intravenösen Infusion vorliegt.

7. Verwendung gemäß einem der Ansprüche 1 bis 4, wobei das Medikament in Form von Kapseln, Pulvern oder Granulaten, in der Form von Tabletten oder Pillen, welche vorzugsweise mit einer enterischen Schicht versehen sind, in der Form ein Zäpfchens, in der Form einer Lösung, einer Suspension oder eines Elixiers oder in der Form von Liposomen vorliegt.

Revendications

1. Utilisation d'un dérivé d'adénine substituée ayant la structure représentée par la formule :



ou d'un sel pharmacologiquement acceptable d'un tel dérivé

formule dans laquelle :
Z représente O^- ou est absent ;
Y représente l'hydrogène ou un substituant contenant de 1 à environ 20 atomes qui est dépourvu de charge ionique nette aux valeurs de pH physiologiques, produit un dérivé soluble de l'adénine et dont la présence sur le motif adénine inhibe la désamination dudit dérivé de l'adénine par l'adénosine-désaminase ; et
X représente l'hydrogène ou fluoro,
étant entendu que lorsque Z est absent, Y ne représente pas l'hydrogène,
pour la préparation d'un médicament pour le traitement de la sclérose en plaques.

2. Utilisation selon la revendication 1, dans laquelle Z est absent et Y est un groupe halogéno.

3. Utilisation selon la revendication 1, dans laquelle ladite adénine substituée est la 2-chloro-2'-déoxyadénosine.

4. Utilisation selon l'une quelconque des revendications 1 à 3, dans laquelle le traitement se fait par application du dérivé de l'adénine au sérum du patient.

5. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle le médicament est sous la forme d'un milieu injectable par voie sous-cutanée.

6. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle le médicament est sous la forme d'une perfusion intraveineuse.

7. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle le médicament est sous la forme de capsules, poudres ou granules, sous la forme de comprimés ou de pilules qui sont éventuellement pourvus d'une couche entérique, sous la forme d'un suppositoire, sous la forme d'une solution, d'une suspension ou d'un élixir ou sous la forme de liposomes.

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Effect of Repeated Treatments with Cladribine (2-Chlorodeoxyadenosine) on Blood Counts in Multiple Sclerosis Patients

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Abstract. We report the results of blood morphology monitoring of 11 remitting-relapsing multiple sclerosis patients who received repeated treatments with cladribine (2-chlorodeoxyadenosine). The drug was given once, daily, subcutaneously (5 mg) or orally (10 mg) for 5 consecutive days, as 6 monthly courses followed by one or two additional courses at 3 or 6 month intervals. The treatments were well tolerated, although many patients suffered from incidental upper respiratory tract infections, most of which occured during the last 6 months of the observation period. One patient had recurrent infections, including an episode of urosepsis. All infections responded to standard therapy with antibiotics. Progressive lymphocyte reduction to 1000/μl on average, and clear but clinically insignificant drop in thrombocytes, was observed. Granulocyte counts were sometimes markedly elevated. A few patients developed macrocytosis, but none required transfusion. With our dosing and schedule, cladribine seems relatively safe in multiple sclerosis patients.

Key words: 2-chlorodeoxyadenosine; blood counts; toxicity.

Introduction

Cladribine (2-chlorodeoxyadenosine, 2-CdA) is a deoxyadenosine analog selectively toxic against human lymphocytes (both resting and proliferating) and monocytes[6,8]. It is also highly active against many leukemia cell lines *in vitro*[1,18]. The drug proved to be a remarkably effective treatment of hairy cell leukemia (response rate > 90% after a single course of treatment)[9,20], and promising results have been reported in other indolent lymphoproliferative diseases, mainly in B cell chronic lymphocytic leukemia (B-CLL) and low grade non-Hodgkin lymphomas[2]. Its immunosuppressive potential has been recognized in an early study when a severe autoimmune hemolytic anemia, concomitant with a diffuse lymphoma, was abated following treatment[7]; this preliminary observation was confirmed in further clinical trials[4]. A recent report[22] suggests that cladribine may favourably influence the natural history of progressive multiple sclerosis, and our preliminary data suggest also some beneficial effect in remitting-relapsing type of this disease[11,23]. Future clinical evaluation of this drug also in other autoimmune disorders seems warranted.

Cladribine displays a marked toxicity toward bone marrow progenitor cells *in vitro*[5,19]. In agreement with these observations, main dose-limiting side effects of the drug in patients with both lymphoid[2] and non-hematological[21] tumors are thrombocytopenia and, less fre-

quently, anemia and granulocytopenia (with consequential infections). Cytopenias, usually more severe in heavily pretreated patients with advanced lymphoid malignancies[1,2], could be attributed to bone marrow involvement in a disease process and/or cumulative toxicity of the drug with previous chemotherapy. However, marrow suppression and prolonged macrocytosis following repeated doses of cladribine was observed also in multiple sclerosis patients, rising concern of a delayed and long lasting hematologic toxicity of the drug even when bone marrow is unaffected[3].

In a pilot study of cladribine in remitting-relapsing multiple sclerosis we have monitored blood morphology of patients taking repeated treatments with the drug over 18 months.

Materials and Methods

Cladribine (purity > 98% by HPLC) was synthesized by Z. Kazimierczuk (Department of Biophysics, University of Warsaw). The drug was prepared as isotonic saline solution, 1 mg/kg for oral use, and 2.5 mg/kg (phosphate-buffered at pH 7.4) for subcutaneous injections. Eleven patients (8 females and 3 males, age 21 – 51, body weight 62 ± 8 SD kg, range 52 – 75 kg) suffering from remitting-relapsing multiple sclerosis, with normal blood counts, and normal kidney and liver function tests, were treated with multiple courses of the drug. Each course consisted of 5 daily doses, 10 mg orally in seven patients and 5 mg subcutaneously in the remaining four. The patients received 6 monthly courses, and additional courses were given at 9, and (in some patients) also at 12 or 15 months. Blood morphology was monitored before each treatment course, and later every three months. Statistical significance of deviations from control values was assessed by *t*-test for paired data and assumed to be statistically significant if $p < 0.05$.

Results

The treatments were well tolerated. Although two study participants taking cladribine orally complained of upper abdominal pain, its relationship to the treatment cannot be ascertained, since none of a considerable number of patients treated with cladribine given orally for B-CLL reported such complaints (J. Liliemark, Department of Clinical Pharmacology, Karolinska Hospital, Stockholm, personal communication). Several patients reported increased frequency of upper respiratory tract infections, most of which occurred during the last 6 months of the observation period. One patient suffered from recurrent infections, including one episode of urinary tract infection which occured during the third month of the study. All infections were succesfully treated with standard antibiotic therapy.

Averaged hematological data of all patients are displayed in the graphs as means ± 1 SEM. Following treatments, lymphocyte counts decreased sharply, reaching the nadir after the sixth course (Fig. 1).

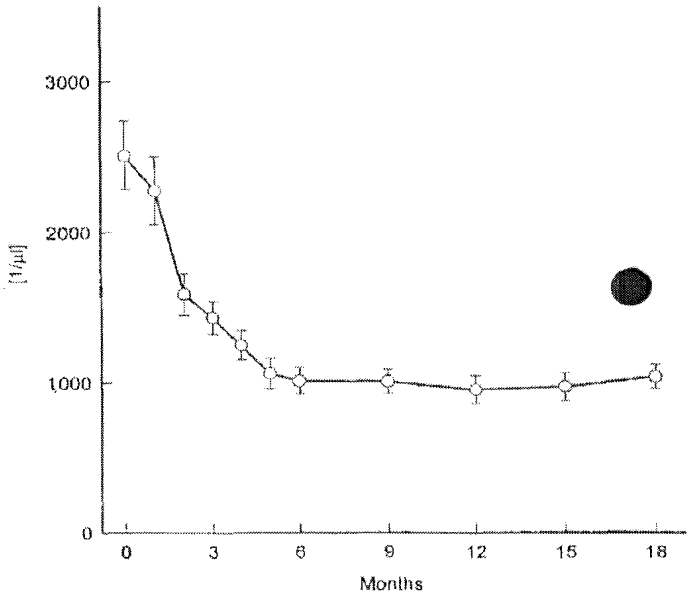

Fig. 1. Average lymphocyte counts during treatment with cladribine. The decrease is statistically significant from the second month of therapy

The degree of lymphocytopenia (its measure being the nadir of lymphocyte counts after 6 months of the study) was not related to the dose of the drug per body weight (Fig. 2), which may reflect differing individual susceptibility of lymphocytes to the cytotoxic effect of the drug. There was no appreciable drop in hemoglobin content (data not shown). Platelet counts decreased significantly (Fig. 3), but never dropped below 100 000/µl. A slight macrocytosis developed in some patients, but was not significant in the whole group, except at 18 months (Fig. 4). Averaged granulocyte count did not significantly deviate from the control level, except of the drop at 18 months (Fig. 5), but transient granulocytosis appeared in some patients (Figs. 6, 7).

Discussion

Until recently 2-chlorodeoxyadenosine had been given by continuous 7-day intravenous infusions and repeated monthly, if required. This mode of admini-

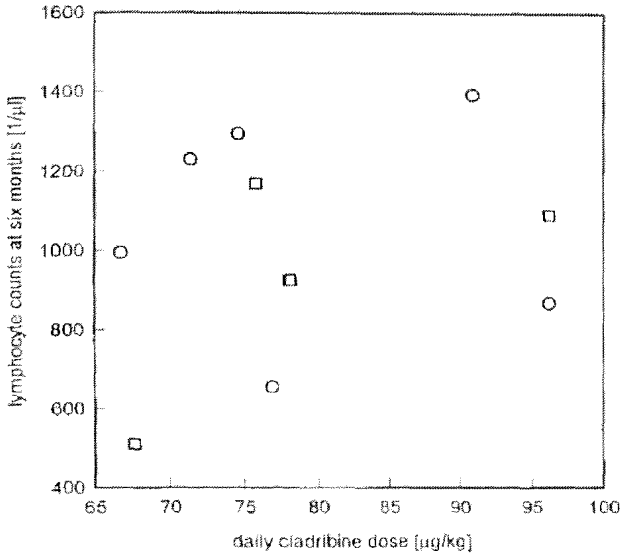

Fig. 2. The lack of correlation between the lymphocyte counts at 6 months of therapy and daily cladribine dose per kg body weight. Squares – the drug taken subcutaneously; circlets – drug taken orally (dose normalized to subcutaneous route assuming 50% oral bioavailability). Data available for 10 patients

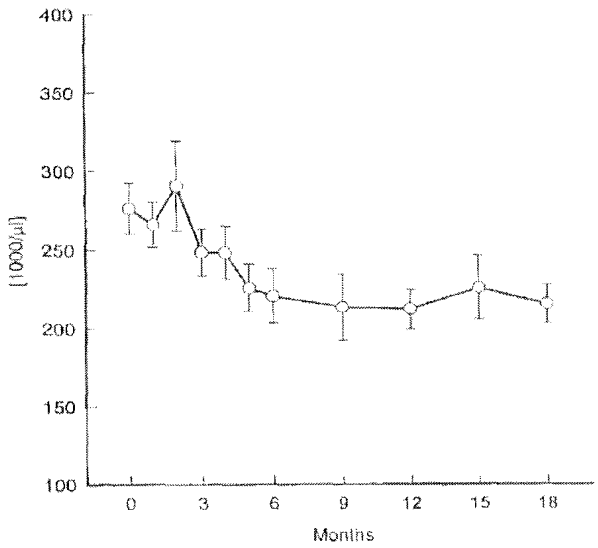

Fig. 3. Average platelet counts during treatment with cladribine. The decrease is statistically significant from the third month of therapy

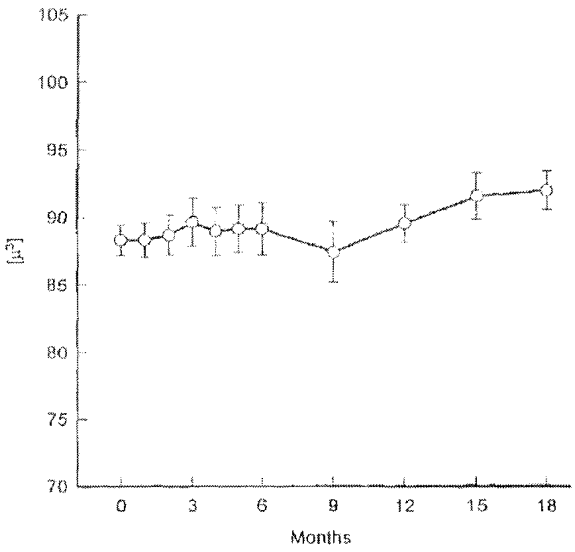

Fig. 4. Average MCV during treatment with cladribine. The rise is statistically significant at 18 months

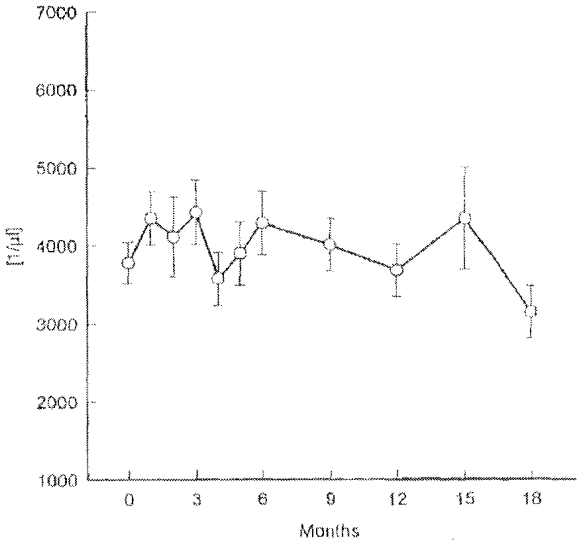

Fig. 5. Average granulocyte counts during treatment with cladribine. The change is statistically significant only at 18 months

stration was established on the basis of the *in vitro* studies suggesting that multi-day exposure to nanomolar concentrations of the drug is required to kill resting human peripheral blood lymphocytes[8]. In this mode of administration a single course of 0.09 mg/kg daily (i.e., total dose 0.63 mg/kg) is usually well tolerated by leukemia patients[2,4], but the probability of cytopenia increases when treatments are repeated.

Recent pharmacokinetic studies have shown that the area under the plasma concentration versus time curve (AUC), which is a measure of the exposure of target cells (peripheral blood lymphocytes) to the drug, is practically of the same size when the dose is given as continuous 24 h i.v. infusion, and when it is given once daily, either as an i.v. infusion lasting 2 h, or subcutaneously, or when a doubled dose is given orally (oral bioavailability is approximately 50%)[14,15]. In the treatment of lymphoid malignancies intermittent dosing schedules proved to be at least as effective as week-long intravenous infusions[13].

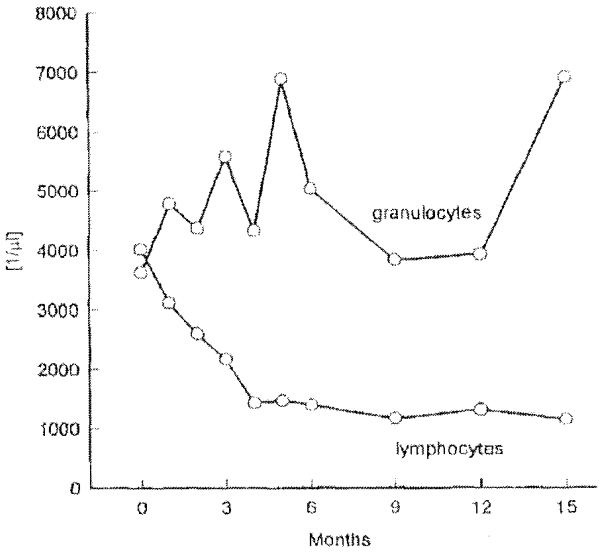

Fig. 6. Lymphocyte and granulocyte counts of the patient no. 9 (a female, the drug given subcutaneously, daily dose 0.07 mg/kg)

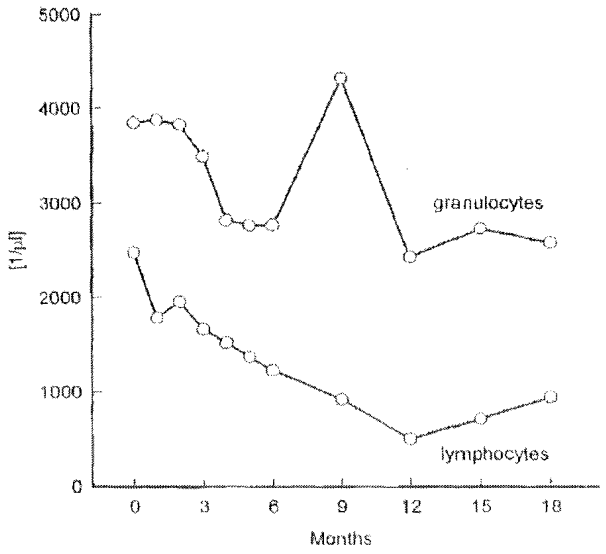

Fig. 7. Lymphocyte and granulocyte counts of the patient no. 4 (a female, the drug given orally, daily dose 0.18 mg/kg)

Cladribine is a pro-drug requiring intracellular enzymatic phosphorylation to exert cytotoxicity[8]. In B-CLL patients at the end of 2 h i.v. infusion intracellular concentration of CdA-phosphates in lymphocytes was two orders of magnitude higher than the peak plasma level of the drug. Its decay had $t_{1/2}$ of approximately 24 h, and lymphocytic intracellular AUC's of CdA phosphates after intermittent and continuous infusion were similar[16,17]. Although no data are available on the intracellular kinetics of CdA-phosphates in non-leukemic lymphocytes *in vivo*, one may expect it to be similar to that in B-CLL cells. Therefore, intermittent (e.g., subcutaneous, or oral) dosing of cladribine should also be effective for reduction of lymphocyte population and induction of immunosuppression. The results of the present study suggest, that it may also produce lower hematologic toxicity.

In the Scripps study, chronic progressive multiple sclerosis patients were treated with 4 to 6 monthly courses of cladribine, dose 0.087—0.1 mg/kg per day for 7 days, given as continuous i.v. infusion. The nadir of lymphocyte counts (ca. 500/µl) occurred after the last treatment, and the rebound was slow and incomplete (even during more than 3 years). While severe hematologic toxicity was observed in only 2 out of more than 20 patients and it could have been related to prior or concomitant intake of other marrow-depressing drugs, all patients on average displayed marked signs of marrow toxicity: a progressive, although moderate and reversible fall in hemoglobin concentration, a distinct macrocytosis and a moderate thrombocytopenia (these changes were not reversing after the treatment was discontinued), and a modest, but noticeable drop in granulocyte counts. Those hematological side effects were markedly more severe than these observed in our patients.

The design our present study and that of Scripps[3,22] differ in some points: 1) patients suffered from different form of the disease (in our group it was remitting-relapsing, while in the Scripps study it was chronic progressive multiple sclerosis); 2) while the daily doses of the drug received by our patients (0.067—0.096 mg/kg) were similar, the total dose in our study was lower on average by approximately 30% because the course of treatment lasted 5 instead of 7 days; 3) we used intermittent dosing (once daily orally or subcutaneously) instead of a week-long continuous infusion. We hypothesise that the last factor is the most important one, i.e., that hematological side effects of cladribine depend on its dosing schedule and are more severe when the drug is given in continuous infusion. A possible explanation may be that the entry of cladribine to the marrow cells is slower than to lymphocytes, and reducing exposure to 5 days and utilizing intermittent drug delivery may to some extent spare marrow progenitors, while still acting on lymphocytes. In agreement with this hypothesis are the data of PETZER et al.[19] who showed that the inhibition by cladribine of proliferation of bone marrow progenitors *in vitro* is achieved at high concentrations (> 300 nM) and it develops over a few days. In some of our patients granulocyte counts increased markedly (supposedly as a respons

to infections), which clearly indicates that with our dosing and schedule the drug is toxic neither to granulocytes nor to granulocyte progenitors.

The reduction of lymphocyte counts in our study was on average only about half of that observed in the Scripps study. Would the severity of lymphocytopenia be an index of therapeutic efficiacy of cladribine, one might expect the Scripps protocol be more effective. However, the depletion of lymphocytes, while certainly being a sort of measure of the effect of cladribine on the lymphatic component of the immune system, may not be the only factor contributing to therapeutic activity of this drug in autoimmune diseases. *In vitro* cladribine, acting by a yet unknown mechanism, is a potent inhibitor of lymphocyte activation[10], and this property may well contribute to its immunosuppressive efficiacy *in vivo*. Functional properties of lymphocytes following *in vivo* treatment with the drug remain be described.

References

1. Avery T. L., Reig J. E., Lumm W. C., Harwood F. C., Santana V. M. and Blakley R. L. (1989): Biochemical pharmacology of 2-chlorodeoxyadenosine in malignant human hematopoietic cell lines and therapeutic effects of 2-bromodeoxyadenosine in drug combinations in mice. Cancer Res., **49**, 4972–4978.
2. Beutler E. (1994): New chemotherapeutic agent: 2-chlorodeoxyadenosine. Semin. Hematol., **31**, 40–45.
3. Beutler E., Koziol J. A., McMillan R., Sipe J. C., Romine J. S. and Carrera C. J. (1994): Marrow suppression produced by repeated doses of cladribine. Acta Haematol., **91**, 10–15.
4. Beutler E., Piro L. D., Saven A., Kay A. C., McMillan R., Longmire R., Carrera C. J., Morin P. and Carson D. A. (1991): 2-Chlorodeoxyadenosine (2-CdA). A potent chemotherapeutic and immunosuppressive nucleoside. Leuk. Lymphoma, **5**, 8–14.
5. Bilgeri R., Petzer A. L., Zilian U., Geisen F. H., Schirmer M., Haun M. and Konwalinka G. (1993): Effect of deoxycytidine on 2-chlorodeoxyadenosine-mediated growth inhibition of normal human erythroid and myeloid progenitor cells. Exp. Hematol., **21**, 432–437.
6. Carrera C. J., Terai C., Lotz M., Curd J. G., Piro L. D., Beutler E. and Carson D. A. (1990): Potent toxicity of 2-chlorodeoxyadenosine against human monocytes *in vitro* and *in vivo*. J. Clin. Invest., **86**, 1480–1488.
7. Carson D. A., Wasson D. B. and Beutler E. (1984): Antileukemic and immunosuppressive activity of 2-chloro-2'-deoxyadenosine. Proc. Natl. Acad. Sci. USA, **81**, 2232–2236.
8. Carson D. A., Wasson D. B., Taetle R. and Yu A. (1983): Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. Blood, **62**, 737–743.
9. Estey E. H., Kurzock R., Kantarjian H. M., O'Brien S. M., McCredie K. B., Beran M., Koller C., Keating M. J., Hirsch-Ginsberg C., Huh Y. O., Stass S. and Freireich E. (1992): Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine (2-CdA). Blood, **79**, 882–887.
10. Górski A. and Grieb P. (1994): Cladribine (2-chloro-2'-deoxyadenosine): new perspectives in clinical immunosuppression. Arch. Immunol. Ther. Exp., **42**, 11–12.
11. Grieb P., Ryba M., Stelmasiak Z., Nowicki J., Solski J. and Jakubowska B. (1994): Cladribine treatment of multiple sclerosis. Lancet, **344**, 538.
12. Hickish T., Serafinowski P., Cunningham D., Oza A., Dorland E., Judson I., Millar B. C., Lister T. A. and Roldan A. (1993): 2-chlorodeoxyadenosine: Evaluation of a novel predominantly lymphocyte-selective agent in lymphoid malignancies. Br. J. Cancer, **67**, 139–143.
13. Juliusson G. and Liliemark J. (1994): Survival of previously treated patients with chronic lymphocytic leukemia following i.v. cladribine (2-chlorodeoxyadenosine, CdA). Abstract. Blood, **84** (suppl. 1), 462.
14. Liliemark J., Albertioni F., Hassan M. and Juliusson G. (1992): On the bioavailability of oral and subcutaneous 2-chloro-2'-deoxyadenosine in humans: alternative routes of administration. J. Clin. Oncol., **10**, 1514–1518.
15. Liliemark J. and Juliusson G. (1991): On the pharmacokinetics of 2-chloro-2'-deoxyadenosine in humans. Cancer Res., **51**, 5570–5572.
16. Liliemark J. and Juliusson G. (1994): 2-Chloro-2'-deoxyadenosine – clinical, biochemical and pharmacokinetic considerations. Arch. Immunol. Ther. Exp., **42**, 7–10.
17. Liliemark J., Petterson B. and Juliusson G. (1991): The relationship between plasma 2-chloro-2'-deoxyadenosine (CdA) and cellular CdA nucleotides (CdAN) after intermittent and continuous i.v. infusion in patients with chronic lymphocytic leukemia. Abstract. Blood, **78** (suppl. 1) 33.
18. Nagourney R. A., Evans S. S., Messenger J. C., Zhuang Su Y. and Weisenthal S. M. (1993): 2-chlorodeoxyadenosine activity and cross resistance patterns in primary cultures of human hematologic neoplasms. Br. J. Cancer, **67**, 10–14.
19. Petzer A. L., Bilgeri R., Zilian U., Haun M., Geisen F. H., Pragnell I., Braunsteiner H. and Konwalinka G. (1991): The inhibitory effect of 2-chlorodeoxyadenosine on granulocytic, erythroid and T lymphocytic colony growth. Blood, **78**, 2583–2587.
20. Piro L. D., Carrera C. J., Carson D. A. and Beutler E. (1990): Lasting remissions in hairy cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. N. Engl. J. Med., **322**, 1117–1121.
21. Saven A., Kawasaki H., Carrera C. J., Waltz T., Copeland B., Zyroff J., Kosty M., Carson D. A., Beutler E. and Piro L. D. (1993): 2-Chlorodeoxyadenosine dose escalation in nonhematologic malignancies. J. Clin. Oncol., **11**, 671–678.
22. Sipe J., Romine J. S., Koziol J. A., McMillan R., Zyroff J. and Beutler E. (1994): Cladribine treatment of chronic progressive multiple sclerosis. Lancet, **344**, 9–13.
23. Stelmasiak Z., Solski J., Nowicki J., Jakubowska B., Ryba M. and Grieb P. (1994): Short term effects of 2-chlorodeoxyadenosine (2-CdA) in remitting-relapsing multiple sclerosis (MS). Abstract. J. Neurol., **241** (suppl. 1), 90.

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Synthesis of 2′-Deoxytubercidin, 2′-Deoxyadenosine, and Related 2′-Deoxynucleosides via a Novel Direct Stereospecific Sodium Salt Glycosylation Procedure

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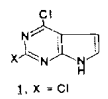
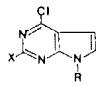
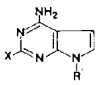
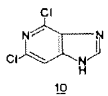
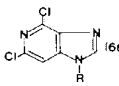
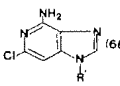
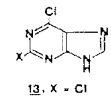
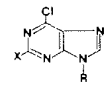
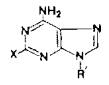
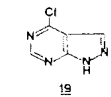
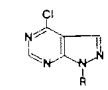
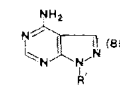
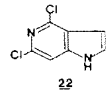
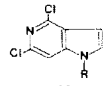
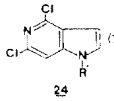
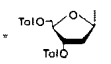
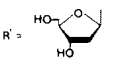
Abstract: A general and stereospecific synthesis has been developed for the direct preparation of 2′-deoxy-β-D-ribofuranosylpurine analogues including 2′-deoxyadenosine derivatives. The reaction of the sodium salt of 4-chloropyrrolo[2,3-*d*]pyrimidine (**4**) or 2,4-dichloropyrrolo[2,3-*d*]pyrimidine (**1**) with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**25**) provided the corresponding *N*-1,2′-deoxy-β-D-ribofuranosyl blocked derivatives (**5** and **2**) which, on ammonolysis, gave 2′-deoxytubercidin (**6**) and 2-chloro-2′-deoxytubercidin (**3**), respectively, in good yield. This glycosylation also readily proceeds in the presence of a 2-methylthio group. Application of this glycosylation procedure to 4,6-dichloroimidazo[4,5-*c*]pyridine (**10**), 6-chloropurine (**16**), 2,6-dichloropurine (**13**), and 4-chloropyrazolo[3,4-*d*]pyrimidine (**19**) gave 2-chloro-2′-deoxy-3-deazaadenosine (**12**), 2′-deoxyadenosine (**18**), 2-chloro-2′-deoxyadenosine (**15**), and 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo-[3,4-*d*]pyrimidine (**21**), respectively. Similarly, glycosylation and ammonolysis of 4,6-dichloro-1*H*-pyrrolo[3,2-*c*]pyridine (**22**) gave 4,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]pyridine (**24**). This stereospecific attachment of the 2-deoxy-β-D-ribofuranosyl moiety appears to be due to a Walden inversion at the C-1 carbon of **25**.

A simple and stereospecific synthesis has now been developed for the direct preparation of 2′-deoxyadenosine derivatives and related analogues. This procedure appears to be of general utility and overcomes many of the limitations found in earlier glycosylation procedures. Derivatives of 2′-deoxyadenosine are of considerable current interest due to the potent antitumor effects of 2-chloro-2′-deoxyadenosine (**15**)[1-4] and 2-bromo-2′-deoxyadenosine.[4] Montgomery has recently found[4] that **15**, given on a frequent schedule, results in 60% survivors of mice injected with L1210 leukemia.

Prior glycosylation procedures introducing the 2-deoxy-β-D-ribofuranosyl (2-deoxy-β-D-*erythro*-pentofuranosyl) moiety into an aglycon reported from our laboratory[5-8] and by others[9-12] invariably provide anomeric mixtures as well as positional isomers which result in very low yields of the desired 2′-deoxynucleoside. In view of these difficulties, a four-step deoxygenation procedure using phenoxythiocarbonylation[13-15] or imidazolylthiocarbonylation[16,17] of the 2′-hydroxy group of the corresponding 3′, 5′-protected β-D-ribonucleoside has recently been developed to provide the requisite 2′-deoxynucleoside. These latter procedures, however, require the availability of the preformed ribonucleoside and unfortunately are not applicable in the presence of haloheterocyclic derivatives,[18] which are most useful for further nucleophilic displacement. We have recently employed the sodium salt of 4,6-dichloro-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine and 1-bromo-2,3,5-tri-*O*-benzoyl-D-ribofuranose in dioxane to give a 68% yield of 4,6-dichloro-2-(methylthio)-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine.[19] Application of this simple single phase sodium salt glycosylation procedure to the synthesis of 2′-deoxynucleosides of chloropurines and related chloropurine analogues is the subject of the present report.

In the present work we elected to use chloroheterocyclic derivatives for glycosylation studies to obtain the corresponding glycosyl intermediates, which could readily be converted into the desired 2′-deoxyadenosine analogues and related derivatives by direct nucleophilic displacement. Treatment of pyrrolo[2,3-*d*]-pyrimidine-2,4(1*H*,3*H*)-dione[20] with $POCl_3$ in the presence of *N*,*N*-dimethylaniline gave 2,4-dichloropyrrolo[2,3-*d*]pyrimidine (**1**). The sodium salt of **1**, produced in situ by NaH in acetonitrile, was treated with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose[21] (**25**) at 50 °C. A clean reaction mixture was obtained, and the product was purified on a silica gel column to give 2,4-dichloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-

Table I. Condensation of the Sodium Salt of Various Chloropurines and Chloropurine Analogues with 1-Chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (25)

starting heterocycle	intermediate (% yield)	final product (% yield)
 1, X = Cl; 4, X = H; 7, X = SCH_3	 2, X = Cl (60); 5, X = H (71); 8, X = SCH_3 (66)	 3, X = Cl (61); 6, X = H (85); 9, X = SCH_3 (72)
 10	 (66) 11	 (66) 12
 13, X = Cl; 16, X = H	 14, X = Cl (59); 17, X = H (59)	 15, X = Cl (71); 18, X = H (78)
 19	 (32) 20	 (85) 21
 22	 (82) 23	 (71) 24
	R = 	R′ = 

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(1) Plunkett, W.; Nowak, B. J.; Robins, R. K. *Proc. Am. Assoc. Cancer Res.* **1980**, *71*, 302.

0002-7863/84/1506-6379$01.50/0

pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**2**) in 60% yield. When **2** was treated with methanolic ammonia at 100 °C for 12 h, deprotection of the sugar with concomitant nucleophilic displacement of the 4-chloro function to an amino group occurred to give 2-chloro-4-amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2-chloro-2′-deoxytubercidin, **3**). Dehalogenation of **3** with Pd/C in a hydrogen atmosphere readily provided 4-amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2′-deoxytubercidin, **6**). 2′-Deoxytubercidin (**6**) was also prepared in good yield by the direct glycosylation of the sodium salt of 4-chloropyrrolo[2,3-*d*]pyrimidine[22] (**4**) with **25** to obtain 4-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**5**), followed by ammonolysis.

This glycosylation was also found to proceed with equal ease in the presence of a methylthio group. Thus, the glycosylation of the sodium salt of 2-(methylthio)-4-chloropyrrolo[2,3-*d*]pyrimidine[23] (**7**) with **25** in acetonitrile gave a 66% yield of 2-(methylthio)-4-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**8**). Ammonolysis of **8** readily gave 2-(methylthio)-4-amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**9**). Dethiation of **9** by treatment with Raney nickel furnished yet another route to 2′-deoxytubercidin (**6**), which in all cases was found to be identical with 2′-deoxytubercidin previously reported.[24,25]

The anomeric configuration of the isolated pyrrolo[2,3-*d*]pyrimidine 2′-deoxynucleosides was assigned by ^{1}H NMR spectroscopy. The pattern of the anomeric proton signal was identical with that published for 2′-deoxytubercidin,[25] as well as for other 2′-deoxyribonucleosides.[6] Since the starting 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose[21] has the α-configuration[26] in the solid state, the exclusive formation of the blocked 2′-deoxy-β-nucleosides in the present study is viewed to be due to a direct Walden inversion (S_N2) at the C_1 carbon by the anionic heterocyclic nitrogen.

This type of direct glycosylation via the sodium salt of a preformed pyrrolo[2,3-*d*]pyrimidine in acetonitrile appears to be considerably superior to previously reported glycosylations of this ring system,[27-30] including phase-transfer procedures.[31,32] Our synthesis of 2′-deoxytubercidin provides a unique simple total synthesis starting with a requisite heterocycle which appears to be superior to published multistep procedures[14,25] requiring first the corresponding ribonucleoside as in the recently described six-step synthesis of 2′-deoxysangivamycin[33] from toyocamycin. In view of the current interest in the new pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotics cadequomycin[34-37] and dapiramicin,[38-40] the presently described procedure should provide a direct route to the synthesis of 2′-deoxycadeguomycin and the 2′-deoxy-β-D-ribofuranosyl derivative of dapiramicin.

This general synthetic procedure has been found to be applicable equally well to the preparation of 3-deazapurine 2′-deoxynucleosides. The following represents the direct preparation of the previously unknown 4-amino-6-chloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)imidazo[4,5-*c*]pyridine (**12**). Treatment of the sodium salt of 4,6-dichloroimidazo[4,5-*c*]pyridine[41] (**10**) with **25** in acetonitrile in an inert atmosphere gave crystalline 4,6-dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)imidazo[4,5-*c*]pyridine (**11**). Ammonolysis of **11** with MeOH/NH_3 at elevated temperature and pressure gave **12**. The UV absorption spectrum of **12** was very similar to that of 4-amino-6-chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine[42] and together with the observed triplet for the anomeric proton in the ^{1}H NMR spectrum established the site of glycosylation in **12** as N-1 and the anomeric configuration as β.

In the purine series, reaction of the protected deoxychloro sugar **25** with the sodium salt of 6-chloropurine[43] (**16**) gave a mixture of two nucleosidic products. After silica gel column chromatography, a 59% yield of 6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**17**) and the corresponding N-7 glycosyl isomer in 11% yield were obtained. Subsequent treatment of **17** with MeOH/NH_3 at 100 °C for 12 h resulted in the deprotection of the glycon moiety with concomitant nucleophilic displacement of the 6-chloro function to give 6-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**18**), identical with an authentic sample of 2′-deoxyadenosine.[44] A similar glycosylation of the sodium salt of 2,6-dichloropurine[43] (**13**) with **25** gave a mixture of 2,6-dichloro-9-(2-deoxy-3,5-di-

(2) Results from the National Cancer Institute on 2-chloro-2′-deoxyadenosine (NSC 105014), submitted from our laboratory, show a T/C (treated/control) of 192 at 25 mg/kg daily dose against L1210 leukemia in mice.

(3) Carson, D. A.; Wasson, D. B.; Kaye, J.; Ullman, B.; Martin, D. W., Jr.; Robins, R. K.; Montgomery, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6865.
(4) Montgomery, J. A. *Cancer Res.* **1982**, *42*, 3911.
(5) Robins, M. J.; Bowles, W. A.; Robins, R. K. *J. Am. Chem. Soc.* **1964**, *86*, 1251.
(6) Robins, M. J.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 4934.
(7) Robins, M. J.; Robins, R. K. *J. Org. Chem.* **1969**, *34*, 2160.
(8) Witkowski, J. T.; Fuertes, M.; Cook, P. D.; Robins, R. K. *J. Carbohydr. Nucleosides, Nucleotides* **1975**, *2*, 1.
(9) For a review of the synthesis of 2′-deoxynucleoside, see: (a) Goodman, L. In "Basic Principles in Nucleic Acid Chemistry"; Ts'o, P. O. P., Ed.; Academic Press: New York, 1974; Vol. I, pp 93–208. (b) Moffatt, J. G. In "Nucleoside Analogues: Chemistry, Biology, and Medical Applications"; Walker, R. T., De Clercq, E., Eckstein, F., Eds.; Plenum Press: New York, 1979; pp 71–164.
(10) Mian, A. M.; Khwaja, T. A. *J. Med. Chem.* **1983**, *26*, 286.
(11) Christensen, L. F.; Broom, A. D.; Robins, M. J.; Bloch, A. *J. Med. Chem.* **1972**, *15*, 735.
(12) Wright, G. E.; Dudycz, L. W. *J. Med. Chem.* **1984**, *27*, 175.
(13) Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 932.
(14) Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.
(15) Lessor, R. A.; Leonard, N. J. *J. Org. Chem.* **1981**, *46*, 4300.
(16) Pankiewicz, K.; Matsuda, A.; Watanabe, K. A. *J. Org. Chem.* **1982**, *47*, 485.
(17) Fukukawa, K.; Ueda, T.; Hirano, T. *Chem. Pharm. Bull.* **1983**, *31*, 1842.
(18) Montgomery, J. A. In "Nucleosides, Nucleotides, and their Biological Applications"; Rideout, J. L.; Henry, D. W., Beacham III, L. M.; Eds.; Academic Press: New York, 1983; pp 19–46.
(19) Kazimierczuk, Z.; Revankar, G. R.; Robins, R. K. *Nucleic Acids Res.* **1984**, *12*, 1179.
(20) West, R. A.; Ledich, K.; Hitchings, G. H. British Patent 812 366 1959; *Chem. Abstr.* **1960**, *54*, 5921.
(21) Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777.
(22) Davoll, J. *J. Chem. Soc.* **1960**, *131*.
(23) Noell, C. W.; Robins, R. K. *J. Heterocycl. Chem.* **1964**, *1*, 34.
(24) Seela, F.; Kehne, A. *Liebigs Ann. Chem.* **1983**, *876*.
(25) Robins, M. J.; Muhs, W. H. *J. Chem. Soc., Chem. Commun.* **1976**, *269*.
(26) Bhattacharya, A. K.; Ness, R. K.; Fletcher, H. G., Jr. *J. Org. Chem.* **1963**, *28*, 428.
(27) Tolman, R. L.; Robins, R. K.; Townsend, L. B. *J. Heterocycl. Chem.* **1967**, *4*, 230.
(28) Tolman, R. L.; Robins, R. K.; Townsend, L. B. *J. Am. Chem. Soc.* **1969**, *91*, 2102.
(29) Tolman, R. L.; Tolman, G. L.; Robins, R. K.; Townsend, L. B. *J. Heterocycl. Chem.* **1970**, *7*, 799.
(30) Townsend, L. B.; Tolman, R. L.; Robins, R. K.; Milne, G. H. *J. Heterocycl. Chem.* **1976**, *13*, 1363.
(31) Seela, F.; Winkeler, H.-D. *Carbohydr. Res.* **1983**, *118*, 29.
(32) Winkeler, H.-D.; Seela, F. *J. Org. Chem.* **1983**, *48*, 3119.
(33) Maruyama, T.; Wotring, L. L.; Townsend, L. B. *J. Med. Chem.* **1983**, *26*, 25.
(34) Tanaka, N.; Wu, R. T.; Okabe, T.; Yamashita, H.; Shimazu, A.; Nishimura, T. *J. Antibiot.* **1982**, *35*, 272.
(35) Wu, R. T.; Okabe, T.; Namibiot. **1982**, *35*, 279.
(36) Kondo, T.; Goto, T.; Okabe, T.; Tanaka, N. *Tetrahedron Lett.* **1983**, *24*, 3647.
(37) Beylin, V. G.; Kawasaki, A. M.; Cheng, C. S.; Townsend, L. B. *Tetrahedron Lett.* **1983**, *24*, 4793.
(38) Shomura, T.; Nishizawa, N.; Iwata, M.; Yoshida, J.; Ito, M.; Amano, S.; Koyama, M.; Kojima, M.; Inouye, S. *J. Antibiot.* **1983**, *36*, 1300.
(39) Nishizawa, N.; Kondo, Y.; Koyama, M.; Omoto, S.; Iwata, M.; Tsuruoka, T.; Inouye, S. *J. Antibiot.* **1984**, *37*, 1.
(40) Seto, H.; Otake, N.; Koyama, M.; Ogino, H.; Kodama, Y.; Nishizawa, N.; Tsuruoka, T.; Inouye, S. *Tetrahedron Lett.* **1983**, *24*, 495.
(41) Rousseau, R. J.; Robins, R. K. *J. Heterocycl. Chem.* **1965**, *2*, 196.
(42) May, J. A., Jr.; Townsend, L. B. *J. Chem. Soc., Perkin Trans. 1* **1975**, 125.
(43) Beaman, A. G.; Robins, R K. *J. Appl. Chem.* **1962**, *12*, 432.
(44) Ness, R. K. In "Synthetic Procedures in Nucleic Acid Chemistry"; Zorbach, W. W., Tipson, R. S., Eds.; Interscience: New York, **1968**; Vol. I, pp 183–187.

O-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**14**), 59% yield, and the corresponding N-7 glycosyl isomer (13% yield) which were separated on a silica gel column using toluene:acetone gradient. Further ammonolysis of **14** with MeOH/NH_3 at 100 °C for 5 h readily gave the desired 2-chloro-2′-deoxyadenosine[11] (**15**) in 71% yield.

Although the enzymatic synthesis of 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**21**) has been documented,[45] there is, until the present work, no report of a suitable chemical synthesis of **21**. Application of the sodium salt glycosylation procedure to 4-chloropyrazolo[3,4-*d*]pyrimidine[46] (**19**) gave 4-chloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**20**) as the major product in 32% yield, along with a minor amount of the N-2 positional isomer. Treatment of **20** with MeOH/NH_3 at room temperature gave **21** in 85% yield. Compound **21** was found to be identical in all respects with the nucleoside prepared enzymatically.[45]

The versatility of this stereospecific glycosylation procedure has also been demonstrated with pyrrolo[3,2-*c*]pyridine (3,7-dideazapurine) ring system containing electronegative substituents. When the sodium salt of 4,6-dichloro-1*H*-pyrrolo[3,2-*c*]pyridine[47] (**22**) was allowed to react with **25** in acetonitrile in an inert atmosphere, 4,6-dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]pyridine (**23**) was formed exclusively in 82% yield. Further treatment of **23** with MeOH/NH_3 at elevated temperature and pressure gave a good yield of 4,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]pyridine (**24**). The ^{1}H NMR spectrum of **24** displayed a triplet for the anomeric proton centered at δ 6.41 (peak width 13.31 Hz) indicating the β configuration. The essentially identical UV absorption spectra of **22** and **24** indicated the site of glycosylation in **24** to be N-1.

Selective nucleophilic displacement of the 4-chlorine atom of **24** and the stereospecific attachment of the 2-deoxy-β-D-ribofuranosyl moiety to other hetereocycles by this simple sodium salt procedure are presently under further investigation in our laboratory.

Experimental Section

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were determined at 90 MHz with a JEOL FX 90Q spectrometer. The chemical-shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. Ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Robertson Labs, Florham Park, NJ. Evaporations were carried out under reduced pressure with the bath temperature below 30 °C.

2,4-Dichloropyrrolo[2,3-*d*]pyrimidine (1). A mixture of dry pyrrolo-[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione[20] (8.0 g, 53 mmol), phosphorus oxychloride (80 mL), and freshly distilled *N*,*N*-dimethylaniline (18 mL) was heated under reflux for 2.5 h. The reaction mixture was evaporated to one-third the volume and poured with stirring onto crushed ice (~350 g). The aqueous solution was brought to pH 2–3 with concentrated NH_4OH and extracted with $CHCl_3$ (3 × 200 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to an oil. The oil was purified on a silica gel column (3 × 40 cm) with successively toluene, toluene–$CHCl_3$ (1:1), and $CHCl_3$ to obtain the pure product which, on crystallization (toluene), gave 0.08 g (8.0%) of analytical sample: mp 249 °C; UV λ_{max} (MeOH) 227 nm (ε 30 400), 290 (4200); ^{1}H NMR (Me_2SO-d_6) δ 6.70 (d, 1, C_5H), 7.80 (d, 1, C_6H). Anal. Calcd for $C_6H_3Cl_2N_3$ (188.01): C, 38.33; H, 1.60; N, 22.35; Cl, 37.71. Found: C, 38.31; H, 1.81; N, 22.15; Cl, 37.42.

2,4-Dichloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2). To a suspension of **1** (0.62 g, 3.3 mmol) in dry CH_3CN (25 mL) was added sodium hydride (50% in oil, 0.17 g, 3.6 mmol) and the mixture was stirred at room temperature under a nitrogen atmosphere for 30 min. 1-Chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose[21] (**25**, 1.28 g, 3.3 mmol) was added portionwise with stirring. The reaction mixture was stirred at 50 °C for 2 h before it was filtered to remove a small amount of insoluble material. Evaporation of the filtrate gave an oily residue, which was purified on an open-bed silica gel column (2.5 × 40 cm) using toluene:acetone (9:1, v/v) as the solvent. The homogeneous product was crystallized from EtOH to give 1.08 g (60%) of **2** as needles: mp 164–165 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.38 and 2.42 (2 s, 6, 2 CH_3), 6.74 (t + d, 2, $C_1'H$ and C_5H), 7.32 and 7.96 (m, 9, 2 Ph and C_6H). Anal. Calcd for $C_{27}H_{23}Cl_2N_3O_5$ (540.4): C, 60.00; H, 4.29; N, 7.78. Found: C, 60.06; H, 4.33; N, 7.92.

2-Chloro-4-amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (2-Chloro-2′-deoxytubercidin, 3). A solution of **2** (0.94 g, 1.74 mmol) in methanolic ammonia (saturated at 0 °C, 15 mL) was heated in a steel bomb at 100 °C for 12 h, and the mixture was evaporated to dryness. The residue was dissolved in MeOH (25 mL) and adsorbed onto silica gel (~25 g). Coevaporation with MeOH (3 × 50 mL) gave a dry residue, which was placed on top of a silica gel column (2 × 40 cm). The column was eluted with $CHCl_3$–MeOH gradient. The title compound was eluted at 15% methanolic chloroform and crystallized from water to yield 0.30 g (61%): mp 205 °C; UV λ_{max} (pH 1) 229 nm (ε 18 500), 276 (10 100); UV λ_{max} (pH 7 and 12) 274 nm (ε 10 700); ^{1}H NMR (Me_2SO-d_6) δ 6.40 (t, 1, $C_1'H$, peak width 14 Hz), 6.60 (d, 1, J = 4.0 Hz, C_5H), 7.36 (d, 1, J = 4.0 Hz, C_6H), 7.50 (br, s, 2, NH_2), and other sugar protons. Anal. Calcd for $C_{11}H_{13}ClN_4O_3$ (284.7): C, 46.41; H, 4.60; N, 19.68. Found: C, 46.34; H, 4.69; N, 19.62.

4-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2′-Deoxytubercidin, (6). Method A. To a solution of **3** (0.57 g, 2 mmol) in 80% aqueous 1-propanol (50 mL) containing K_2CO_3 (0.10 g) was added Pd/C (10%, 50 mg) and the mixture was hydrogenated at 2 atm for 2 h. The mixture was filtered through a Celite pad, and the filtrate was evaporated to dryness. Crystallization of the residue from water gave 0.45 g (90%) of 2′-deoxytubercidin: mp 218 °C [lit.[24] mp 216 °C and all other physicochemical properties of **6** are identical with those for 2′-deoxytubercidin previously reported.

Method B. To a solution of **9** (0.59 g, 2 mmol) in 1-propanol (50 mL) was added Raney nickel (W-4, wet weight 4 g) and the mixture was heated under reflux for 5 h. After cooling to room temperature, the mixture was filtered through a Celite pad and the filtrate evaporated to dryness. The residue was crystallized from water to give 0.35 g (70%): mp 218 °C, which was identical in every detail with the 2′-deoxytubercidin **6** prepared by method A.

Method C. In the same manner as for **2**, 4-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**5**) was prepared by using 4-chloropyrrolo[2,3-*d*]pyrimidine[22] (**4**, 1.22 g, 8 mmol), NaH (50% in oil, 0.40 g, 8.3 mmol), **25** (3.1 g, 8 mmol), and CH_3CN (50 mL) to yield 2.9 g (71%): mp 118 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.80 (d + t, overlap, 2, C_5H and $C_1'H$), 7.36 and 7.96 (m, 9, 2 Ph and C_6H), 8.70 (s, 1, C_2H). Anal. Calcd for $C_{27}H_{24}ClN_3O_5$ (505.9): C, 64.10; H, 4.78; N, 8.31. Found: C, 63.95; H, 4.80; N, 8.19.

A solution of **5** (0.20 g, 0.75 mmol) and MeOH/NH_3 (saturated at 0 °C, 12 mL) was heated at 120 °C for 12 h and then evaporated to dryness. The residue was dissolved in water (50 mL), adsorbed on Dowex 1-X8 (OH^-) column (1 × 10 cm) and eluted with water (200 mL), followed by H_2O–MeOH (3:1, v/v, 300 mL) gave 2′-deoxytubercidin, 0.16 g (85%), mp 217–218 °C, and was identical in every detail with **6** prepared by method A.

2-(Methylthio)-4-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3*d*]pyrimidine (8). In the same manner as for **2**, the title compound was prepared by using 2-(methylthio)-4-chloropyrrolo[2,3-*d*]pyrimidine[23] (**7**, 0.60 g, 3 mmol), NaH (50% in oil, 0.16 g, 3.3 mmol), CH_3CN (25 mL), and **25** (1.18 g, 3 mmol). Purification on a silica gel column (2 × 30 cm) with toluene–acetone (95:5, v/v) gave crystalline (from EtOH) compound, 1.10 g (66%): mp 118 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.36 and 2.40 (2 s, 6, 2 CH_3), 6.66 and 6.74 (t + d, 2, $C_1'H$ and C_5H), 7.35 and 7.90 (m, 9, 2 Ph and C_6H). Anal. Calcd for $C_{28}H_{26}ClN_3O_5S$ (552.05): C, 60.92; H, 4.75; N, 7.61. Found: C, 61.16; H, 4.96; N, 7.43.

2-(Methylthio)-4-amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (9). A solution of **8** (2.50 g, 4.5 mmol) in methanolic ammonia (saturated at 0 °C, 50 mL) was heated in a steel bomb at 100 °C for 12 h, and the resulting solution was evaporated to dryness. The residue was purified on a silica gel column (3 × 40 cm) with MeOH–$CHCl_3$ (1:6) as the solvent. Crystallization of the homogeneous product from MeOH gave the title compound, 0.97 g (72%): mp 233–234 °C (lit.[24] mp 233 °C); UV λ_{max} (pH 1) 226 nm (ε 21 300), 280 (14 000); UV λ_{max} (pH 7 and 12) 236 nm (ε 23 100), 282 (14 200) [lit.[24] UV λ_{max} (MeOH) 234 (ε 25 700), 281 (15 000)].

4,6-Dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)imidazo[4,5-*c*]pyridine (11). In the same manner as for **2**, the title compound was prepared by using 4,6-dichloroimidazo[4,5-*c*]-pyridine[41] (**10**, 0.61 g, 3.2 mmol), NaH (50% in oil, 0.17 g, 3.5 mmol),

(45) Stout, M. G.; Hoard, D. E.; Holman, M. J.; Wu. E. S.; Siegel, J. M. *Methods Carbohydr. Chem.* **1976**, *7*, 19.
(46) Robins, R. K. *J. Am. Chem. Soc.* **1956**, *78*, 784.
(47) Schneller, S. W.; Hosmane, R. S. *J. Heterocycl. Chem.* **1978**, *15*, 325.

CH_3CN (25 mL) and **25** (1.38 g, 3.5 mmol). Purification on a silica gel column (4 × 40 cm) with toluene–acetone (95:5, v/v) gave crystalline (from EtOH) product, 1.15 g (66%): mp 165–167 °C; ^1H NMR (Me_2SO-d_6) δ 2.40 and 2.44 (2 s, 6, 2 CH_3), 6.68 (t, 1, $C_1'H$, peak width 14.0 Hz), 7.38 and 7.96 (m, 9, 2 Ph and C_7H), 8.84 (s, 1, C_2H). Anal. Calcd for $C_{27}H_{23}Cl_2N_3O_5$ (540.4): C, 60.00; H, 4.29; N, 7.78. Found: C, 60.25; H, 4.50; N, 7.59.

4-Amino-6-chloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)imidazo[4,5-*c*]pyridine (12). Ammonolysis of **11** (1.30 g, 2.4 mmol) with $MeOH/NH_3$ (50 mL) at 135–140 °C for 25 h in the same manner as described for **3** gave the title compound. Crystallization from water gave analytical sample, 0.45 g (66%): mp 186–187 °C; UV λ_{max} (pH 1) 266 nm (ε19 300), 285 (sh) (13 400); UV λ_{max} (pH 7 and 12) 271 nm (ε 20 600); ^1H NMR (Me_2SO-d_6) δ 6.22 (t, 1, $C_1'H$, peak width 13.0 Hz), 6.66 (s, 2, NH_2), 6.97 (s, C_7H), 8.30 (s, 1, C_2H), and other sugar protons. Anal. Calcd for $C_{11}H_{13}ClN_4O_3$ (284.7): C, 46.41; H, 4.60; N, 19.68. Found: C, 46.40; H, 4.80; N, 19.53.

2,6-Dichloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (14). A mixture of 2,6-dichloropurine[43] (**13**, 0.95 g, 5 mmol) and sodium hydride (50% in oil, 0.25 g, 5.2 mmol) in anhydrous CH_3CN (35 mL) was stirred at ambient temperature under a nitrogen atmosphere for 30 min. Dry, powdered **25** (1.95 g, 5 mmol) was added portionwise with stirring, during 20 min, and stirring was continued for further 15 h. A small amount of insoluble material was removed by filtration. Evaporation of the solvent gave an oil residue, which was purified on a silica gel column (5 × 60 cm) with toluene–acetone (9:1, v/v) as the solvent. The following two nucleosides were isolated in the order listed; the title compound (**14**) was crystallized from EtOH to yield 1.60 g (59%): mp 159–162 °C [lit.[11] mp 155–157 °C].

The N-7 glycosyl isomer 2,6-dichloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine was isolated and crystallized from EtOH to yield 0.35 g (13%): mp 141–143 °C; ^1H NMR (Me_2SO-d_6) δ 6.88 (t, 1, $C_1'H$, peak width 14.5 Hz), 7.36 and 7.90 (m, 8, Ph), 9.28 (s, 1, C_8H). Anal. Calcd for $C_{26}H_{22}Cl_2N_4O_5$ (541.4): C, 57.68; H, 4.09; N, 10.35. Found: C, 57.55; H, 4.00; N, 10.36.

2-Chloro-6-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (15). A solution of **14** (2.50 g, 4.6 mmol) in CH_3OH/NH_3 (saturated at 0 °C, 60 mL) was heated at 100 °C for 5 h, and the mixture was evaporated to dryness. The residue was purified on a silica gel column (5 × 40 cm) with $CHCl_3$–MeOH (8:2, v/v) as the solvent. Crystallization of the homogeneous solid from EtOH gave 0.87 g (71%) of analytically pure title compound: mp 220 °C (softens), resolidifies, turns brown, does not melt below 300 °C [lit.[11] mp 210–215 °C (softens) and then solidifies and turns brown].

6-Chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (17). In the same manner as for **14**, reaction of the sodium salt of 6-chloropurine[43] (**16**, 0.77 g, 5 mmol and 50% NaH in oil, 0.25 g, 5.2 mmol) with **25** (2.0 g, 5.15 mmol) in CH_3CN (50 mL) gave 1.51 g (59%) of crystalline (from EtOH) **17**: mp 107–109 °C; ^1H NMR (Me_2SO-d_6) δ 6.76 (t, 1, $C_1'H$, peak width 14.0 Hz), 7.36 and 7.94 (m, 8, Ph), 8.80 (s, 1, C_2H), 9.00 (s, 1, C_8H). Anal. Calcd for $C_{26}H_{23}ClN_4O_5$ (506.9): C, 61.60; H, 4.57; N, 11.05. Found: C, 61.73; H, 4.72; N, 11.03.

The N-7 glycosyl isomer 6-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine was isolated and crystallized from EtOH to yield 0.29 g (11%): mp 152–153 °C; ^1H NMR (Me_2SO-d_6) δ 6.96 (t, 1, $C_1'H$, peak width 14.5 Hz), 7.36 and 7.94 (m, 8, Ph), 8.94 (s, 1, C_2H), 9.26 (s, 1, C_8H). Anal. Calcd for $C_{26}H_{23}ClN_4O_5$ (506.9): C, 61.60; H, 4.57; N, 11.05. Found: C, 61.55; H, 4.49; N, 11.05.

6-Amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (2′-Deoxyadenosine, 18). A solution of **17** (1.01 g, 2 mmol) in $MeOH/NH_3$ (18 mL) was heated at 100 °C for 12 h and then evaporated to dryness. The aqueous solution of the residue was extracted with $CHCl_3$ (2 × 25 mL), followed by ether (2 × 25 mL), and then evaporated to dryness. The residue was crystallized from water to yield 0.41 g (78%): mp 186–189 °C [lit.[44] mp 187–189 °C, and all other physico-chemical properties of **18** are identical with 2′-deoxyadenosine reported in literature].

4-Chloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrazolo[3,4-*d*]pyrimidine (20). In the same manner as for **2**, the title compound was prepared by using 4-chloropyrazolo[3,4-*d*]pyrimidine[46] (**19**, 1.54 g, 10 mmol), NaH (50% in oil, 0.57 g, 12 mmol) dioxane (100 mL), and **25** (3.9 g, 10 mmol). Purification of the product on a Kieselgel-60 (230–400 mesh) flash column (2.4 × 25 cm) with hexane–ether (4:1, v/v) gave crystalline analytical sample, 1.62 g (32%): mp 130–132 °C; UV λ_{max} (MeOH) 240 nm (ε 17 600); ^1H NMR ($CDCl_3$) δ 2.40 and 2.44 (2 s, 6, 2 CH_3), 6.98 (t, 1, $C_1'H$, peak width 14.0 Hz), 7.27 and 7.96 (m, 8, Ph), 8.16 (s, 1, C_6H), 8.78 (s, 1, C_3H). Anal. Calcd for $C_{26}H_{23}ClN_4O_5$ (506.9): C, 61.60; H, 4.57; N, 11.05; Cl, 6.99. Found: C, 61.49; H, 4.66; N, 10.86; Cl, 7.27.

4-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo[3,4-*d*]pyrimidine (21). In the same manner as for **18**, compound **21** was prepared by using **20** (0.25 g, 0.49 mmol) and $MeOH/NH_3$ (35 mL). After crystallization from aqueous ethanol gave 0.10 g (85%): mp 240–242 °C [lit.[45] mp 245–246 °C, and was identical in all respects with **21** prepared enzymatically].

4,6-Dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]pyridine (23). In the same manner as for **2**, the title compound was prepared by using 4,6-dichloro-1*H*-pyrrolo[3,2-*c*]-pyridine[47] (**22**, 0.40 g, 2.15 mmol), NaH (50% in oil, 0.10 g, 2.4 mmol), CH_3CN (100 mL), and **25** (0.84 g, 2.15 mmol). Purification of the product on a Kieselgel-60 (230–400 mesh) flash column with toluene–EtOAc (7:1, v/v) gave 0.95 g (82%) as colorless foam: UV λ_{max} (EtOH) 227 nm (ε 80 200), 240 (54 100), 274 (14 100); ^1H NMR ($CDCl_3$) δ 2.43 and 2.46 (2 s, 6, 2 CH_3), 6.34 (t, 1, $C_1'H$, peak width 14.3 Hz), 6.60 (d, 1, J = 4.5 Hz, C_3H), 7.30 (m, 6, Ph, C_2H and C_7H), 7.94 (m, 4, Ph). Anal. Calcd for $C_{28}H_{24}Cl_2N_2O_5$ (539.4): C, 62.34; H, 4.48; N, 5.19. Found: C, 62.07; H, 4.53; N, 4.98.

4,6-Dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]-pyridine (24). Compound **23** (0.80 g, 1.5 mmol) was combined with methanolic ammonia (50 mL, saturated at 0 °C) and heated in a steel bomb at 135–150 °C for 20 h. The reaction mixture was evaporated to dryness and the residue was purified on a Kieselgel-60 (230–400 mesh) flash column with CH_2Cl_2–MeOH (14:1, v/v) to obtain 0.32 g (71%) of title compound, which was crystallized from aqueous ethanol as colorless needles: mp 173 °C (softens at 110 °C); UV λ_{max} (pH 1, 7 and 11) 224 nm, (ε 29 300), 274 (5600), 289 (sh) (3600); ^1H NMR (Me_2SO-d_6) δ 6.41 (t, 1, $C_1'H$, peak width 13.31 Hz), 6.67 (d, 1, J = 3.38 Hz, C_3H), 7.88 (d, 1, J = 3.61 Hz, C_2H), 7.95 (s, 1, C_7H), and other sugar protons. Anal. Calcd for $C_{12}H_{12}Cl_2N_2O_3$ (303.1): C, 47.55; H, 3.99; N, 9.24; Cl, 23.39. Found: C, 47.63; H, 4.11; N, 9.06; Cl, 23.18.

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Registry No. 1, 90213-66-4; **2**, 91713-42-7; **3**, 91741-81-0; **4**, 3680-69-1; **5**, 91713-43-8; **6**, 60129-59-1; **7**, 57564-94-0; **8**, 91713-44-9; **9**, 86402-13-3; **10**, 2589-12-0; **11**, 91713-45-0; **12**, 91741-82-1; **13**, 5451-40-1; **14**, 38925-80-3; **15**, 4291-63-8; **16**, 87-42-3; **17**, 91713-46-1; **18**, 958-09-8; **19**, 5399-92-8; **20**, 91713-47-2; **21**, 17318-21-7; **22**, 67139-79-1; **23**, 91713-48-3; **24**, 91713-49-4; **25**, 4330-21-6; pyrrolo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione, 39929-79-8; 2,6-dichloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrine, 91713-50-7; 6-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine, 91713-51-8.

Article abstract—One method of evaluating the degree of neurologic impairment in MS has been the combination of grades (0 = normal to 5 or 6 = maximal impairment) within 8 Functional Systems (FS) and an overall Disability Status Scale (DSS) that had steps from 0 (normal) to 10 (death due to MS). A new Expanded Disability Status Scale (EDSS) is presented, with each of the former steps (1,2,3 . . . 9) now divided into two (1.0, 1.5, 2.0 . . . 9.5). The lower portion is obligatorily defined by Functional System grades. The FS are Pyramidal, Cerebellar, Brain Stem, Sensory, Bowel & Bladder, Visual, Cerebral, and Other; the Sensory and Bowel & Bladder Systems have been revised. Patterns of FS and relations of FS by type and grade to the DSS are demonstrated.

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Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS)

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In 1955 I described "a new scale for evaluating disability in multiple sclerosis,"[1] later known as the Disability Status Scale (DSS), devised to evaluate isoniazid as a possible treatment.[2] This scale was also used in the first multicentered, randomized, placebo-controlled, double-blind trial of MS therapy,[3] which refuted our original claim, a decision with which we had to concur from our later experience.[4] The DSS had 10 grades or steps beyond 0 (normal), extending to status 10 (death due to MS). The scale was "intended to measure the maximal function of each patient as limited by . . . neurologic deficits,"[1] and it was based on neurologic examination.

The DSS was later made half of a bifid rating system, the other part "being a series of grades in each of eight functional groupings. . . . In each portion, there is a numerical rating which is mutually exclusive in its category, and the higher the number, the greater is the dysfunction. Only objectively verifiable defects due to multiple sclerosis as elicited upon neurologic examination are included. Symptoms are discarded."[5]

The functional groups, later called Functional Systems (FS), were Pyramidal (P), Cerebellar (Cll), Brain Stem (BS), Sensory (S), Bowel & Bladder (BB), Visual (V), Cerebral or Mental (Cb), and Other or Miscellaneous (O) Functions. All save the last were graded from 0 (normal) to maximal impairment (grade 5 or 6); the "Other" FS was dichotomous, with 0 as none and 1 as any present. Approximate equivalents for the DSS steps were also provided. The Functional Systems were mutually exclusive in terms of neuroanatomy, but together comprised all neurologic abnormalities on examination that can be attributed to MS lesions. The FS were not additive; each FS could be compared over time only with itself, and for this reason it was necessary to retain the DSS for overall comparisons of the same patient at different examinations.

The FS were modified in 1965 by changing the Sensory scale from 0-5 to 0-6 and redefining the upper grades for Bowel & Bladder.[6] As will be seen below, the Sensory System is again being revised, and Bowel & Bladder has a new step.

This two-part system of assessing neurologic impairment in MS has been used in several studies, and it has been proposed for adoption as one part of a tridimensional scheme for a "minimal data set" in MS, which will be discussed below. However, some investigators believe the DSS is too insensitive to change in the middle ranges, and have urged division of step 7 into two parts. Further, while the DSS was considered satisfactory in several treatment trials in acute bouts, it was thought that there should be more

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room for change in studies of chronic MS.

For these reasons, an Expanded DSS (EDSS) is now presented. It provides, for each step from 1 through 9, two steps that together add up to the same step of the original DSS. This division relies even more heavily on the standard neurologic examination as encoded in the Functional Systems. In fact, it is fully defined in the lower ranges by the FS grades. For this reason, before presenting the Expanded DSS, we need to consider the Functional Systems.

Functional Systems. The grades for each of the Functional Systems are defined in appendix A. They are identical with those provided in 1965[6] except for the new Sensory and Bowel & Bladder Systems. The frequency of involvement in each system at admission to the hospital for an early bout of MS in one series is described in table 1.[7]

Recall that each FS is independent of the others, yet together they reflect all neurologic impairment in MS. There are over 1.3 million possible patterns of involvement by FS type and grade. However, if we consider each System as just involved (1) or not involved (0), then neurologic impairment can be defined by an eight-digit binary number. For example, a patient with Pyramidal, Cerebellar, and Sensory signs, the other Systems normal, would be described as 1101 0000. There are then only 256 possible patterns (2^8) into which a patient can fall. From the same series as in table 1, there are described the most common patterns to be expected if lesions in one system were independent of lesions in the others (table 2). These expected frequencies compare well with those actually observed for the same specific patterns.[8] One-half of the patients fell into one of only 14 patterns, and ¼ into one of only 4 patterns.

Several points of clarification may be in order for the Functional Systems. Pyramidal, Cerebellar, Sensory, and Bowel & Bladder functions all refer to impairment of body parts below the head *only* (regardless of the site of the lesions), and Brain Stem functions have always referred to impairment "attributable to lesions of supra- and intersegmental tracts subserving cranial nerves 3 through 12, together with involvement of these nuclei or their intramedullary fibers. These, therefore ... encompass pseudobulbar palsies and scanning speech ... in addition to the so-called cranial nerve functions."[5]

For each FS and the DSS, the rule remains: "Where criteria for the precise grade are not met, the nearest appropriate category is utilized."[5] Thus Pyramidal grade 5 would be used rather than 4 for one who is almost paraplegic. Whatever the specific grade definition, then, "almost" or "practically" can be prefixed. One method for difficult decisions is to "bracket" the likely grade and then cone down on the most applicable.

The Expanded Disability Status Scale. The EDSS (appendix B) will be discussed under con-

Table 1. Percentage frequency of involvement according to Functional Systems (FS) from neurologic examinations at admission to hospital for an early bout of MS; Army WW II series*

Functional Systems (FS)	% involved	Total N known
Pyramidal (P)	84.9	511
Cerebellar (Cll)	76.9	481
Brain Stem (BS)	73.0	514
Sensory (S)	55.2	478
Bowel & Bladder (BB)	22.6	517
Visual† (V)	33.9	425
Cerebral-total‡ (Cb)	20.7	487
Cerebral-mentation§	2.9	487
Other (O)	14.9	523

* From Kurtzke et al, Acta Neurol Scand 1972;48:19-46.
† Neuropathic signs either/both eyes; see *.
‡ Includes mood changes only (step 1).
§ Steps 2+ on the scale.

Table 2. Patterns of involvement by Functional System (FS) from neurologic examinations at admission to hospital for an early bout of MS; Army WWII series*

Rank†	Pattern‡	No. of cases O	No. of cases E	Cumulative p§ O	Cumulative p§ E
1	1111 0000	31	28.92	0.093	0.086
2	1110 0000	29	28.74	0.179	0.172
3	1111 0100	12	15.32	0.215	0.218
4	1110 0100	16	15.23	0.263	0.263
5	1101 0000	14	9.37	0.304	0.291
6	1100 0000	8	9.32	0.328	0.319
7	1011 0000	6	8.78	0.346	0.345
8	1010 0000	7	8.72	0.367	0.371
9	1111 0010	15	7.78	0.412	0.395
10	1110 0010	8	7.73	0.436	0.418
11	1111 1000	11	7.09	0.469	0.439
12	1110 1000	4	7.05	0.481	0.460
13	0111 0000	1	6.18	0.484	0.478
14	0110 0000	9	6.15	0.510	0.497
15-256	all other	164	168.63	1.000	1.000
256	Total	335	335.01	1.000	1.000

* Adapted from Kurtzke, Acta Neurol Scand 1970;46:493-512.
† Rank order of expected frequency of specific pattern, based upon product of individual observed frequencies with hypothesis of independence for all patterns where E ≥ 5.0; O = observed and E = expected frequency. $\chi^2_{14} = 20.58$, $p > 0.10$ for O versus E.
‡ Involved (1) or not involved (0) for P, Cll, BS, S, BB, V, Cb, O in cited order; cases with complete information on all 8 FS.
§ Cumulative proportion (p) of total, observed (O), and expected (E) patterns.

Table 3. Percentage frequency distribution of Functional System (FS) grades according to DSS steps. I: DSS 1-2*

DSS 1-2 FS	FS grades 0	1	2	3	4	5	6	100% = (N)
	(percentages)							
P	51.7	35.3	13.0	—	—	—	—	(207)
Cll†	65.8	14.1	20.1	—	—	—	NA	(199)
BS	48.5	29.9	14.7	6.9	—	—	NA	(204)
S‡	79.6	8.9	11.5	—	—	—	NA	(191)
BB‡	90.7	6.4	2.9	—	—	—	NA	(204)
V§	68.2	18.2	13.6	—	—	—	—	(22)
Cb	91.9	7.6	---------0.5---------		—	—	NA	(198)
O	86.6	13.4	NA	NA	NA	NA	NA	(307)

* Data from some 2,000 exams in 20 years among 527 males, Army WWII series.
† Excludes those with Pyramidal grade 3+.
‡ 1961 scales.
§ VA Hospital series (N = 392).
— No cases.
NA Not applicable; step(s) not in scale.

secutive groupings of the original DSS. For this expansion, we have had to make more finite and arbitrary distinctions than in the original scale.

DSS Step 0. As before, this defines the normal neurologic examination—regardless of symptoms. Therefore, all FS are grade 0, except for Cerebral System grade 1. Cerebral "grade 1 refers to mood aberrations such as euphoria or depression, which may not be a primary effect of the disease process, but this is hoped to represent that stage of brain damage when alterations of personality or emotional control are the sole features."[5] For DSS step 0 and step 1, Cerebral grade 1 is treated as a 0.

DSS Steps 1-2. These steps refer to minimal objective abnormality, with step 1 as signs without impaired function. Table 3 shows the distribution of FS grades for DSS 1-2 from an overview of some 20 years' follow-up examinations in 527 men with MS, our Army WW II series.[8] The ratio of step 2 to 1 was about 2:1. The DSS scores in this series were not strictly delimited by the FS equivalents described here. Nevertheless, the low frequency of involvement is evident; this was essentially limited to FS grades 1 and 2 except for the 7% in Brain Stem grade 3. The FS scales used here and below are the 1961 variants for Sensory and Bowel & Bladder.

EDSS Step 1.0 is limited to one FS grade 1, excluding Cerebral grade 1, with all others grade 0.

EDSS Step 1.5 is defined as two or more FS grade 1, again excluding Cerebral grade 1, but no grade above 1 in any FS.

EDSS Step 2.0 is limited to one FS grade 2, others grade 0 or 1.

EDSS Step 2.5 is limited to two FS grade 2, others grade 0 or 1.

Note that it is irrelevant which FS are involved, and from table 3, it is likely to be any of them except Bowel & Bladder or Cerebral.

DSS Steps 3-4. These steps still refer to mild disorder, not sufficient to impede normal activities of daily living or work in most situations. However, a concert pianist, a pilot, or a steeplejack would doubtless not be able to function as usual and still be ascribable to these steps. Full ambulation—meaning ability to be up and about all day and to walk usual distances without resting—characterize these steps. Impaired ambulation of any degree should not occur with FS grades defining DSS step 3. There is some overlap of FS in steps 4 and 5. Table 4 delineates the distribution of FS grades for DSS 3-4. The ratio of step 3 to 4 was about unity. Only rarely was grade 4 attained. We begin to see the predominance of Pyramidal involvement, closely followed by Cerebellar and Brain Stem.

EDSS Step 3.0 is limited to one FS grade 3, or three or four FS 2, others being 0 or 1.

EDSS Step 3.5 is limited to one FS grade 3 plus one or two grade 2, or two FS grade 3, or five FS grade 2, others being grade 0 or 1.

EDSS Step 4.0 consists of combinations just exceeding two grade 3, or one grade 3 plus two grade 2, or five grade 2; or one FS grade 4 alone, all others being grade 0 or 1. At this point, the ambulation/work/daily activity abilities start to take precedence over the precise FS grades. With FS that exceed the criteria for EDSS step 3.5, there must be, for step 4.0, full ambulation (including ability to walk without aid or rest for some 500 meters), and ability to carry out full daily activities to include work of average physical difficulty.

EDSS Step 4.5 has the same minimal FS grade requirements as step 4.0. The patient must be able to walk without aid or rest for some 300 meters and to work a full day in a position of average difficulty. The patient is up and about most of the day, but some limitation of full activity separates this from step 4.0.

DSS Steps 5-6. The patient is not ordinarily housebound and can walk. Seldom is a full work day possible without special provisions. The original DSS 5 was defined as "maximal motor function

Table 4. Percentage frequency distribution of Functional System (FS) grades according to DSS steps. II: DSS 3-4*

DSS 3-4 FS	FS grades 0	1	2	3	4	5	6	100% = (N)
				(percentages)				
P	18.5	19.9	25.4	35.1	1.1	—	—	(664)
Cll†	26.5	11.6	45.1	16.9	—	—	NA	(623)
BS	29.8	27.1	23.9	19.0	0.2	—	NA	(652)
S‡	49.4	6.5	31.7	12.2	--------0.2--------		NA	(596)
BB‡	77.4	7.1	11.8	3.7	—	—	NA	(650)
V§	60.6	2.8	12.8	9.2	----------4.6----------			(109)
Cb	80.3	16.8	----------2.9--------		—	—	NA	(615)
O	84.8	15.2	NA	NA	NA	NA	NA	(784)

* Data from some 2,000 exams in 20 years among 527 MS males, Army WWII series.
† Excludes those with Pyramidal grade 3+.
‡ 1961 scales.
§ VA Hospital series (N = 392).
— No cases.
NA Not applicable; step(s) not on the scale.

Table 5. Percentage frequency distribution of Functional System (FS) grades according to DSS steps. III: DSS 5-6*

DSS 5-6 FS	FS grades 0	1	2	3	4	5	6	100% = (N)
				(percentages)				
P	2.1	6.6	8.5	49.5	32.8	0.5	—	(424)
Cll†	5.6	2.5	24.9	56.7	10.3	—	NA	(358)
BS	19.2	23.1	30.2	26.0	1.5	—	NA	(407)
S‡	29.8	7.2	40.3	22.4	----------0.3--------		NA	(362)
BB‡	59.3	10.5	17.1	10.1	1.0	2.0	NA	(398)
V§	60.8	6.4	15.2	11.2	----------6.4----------			(125)
Cb	72.6	20.7	----------5.0----------		0.8	0.8	NA	(362)
O	72.1	27.9	NA	NA	NA	NA	NA	(459)

* Data from some 2,000 exams in 20 years among 527 MS males, Army WWII series.
† Excludes those with Pyramidal grade 3+.
‡ 1961 scales.
§ VA Hospital series.
— No cases.
NA Not applicable; step(s) not in scale.

walking unaided up to several blocks," and for 6 it was "assistance required for walking."[1] There is generally some impairment in usual daily activities. Table 5 indicates for these steps the increasing frequency and severity of FS involvement, particularly Pyramidal and Cerebellar systems, with Brain Stem and Sensory not far behind. The ratio of step 5 to 6 was about 1.7:1. The principal discrimination among these four new EDSS steps rests with walking; the patient's statements about walking are ordinarily acceptable, but direct observation—and on more than one occasion—may be required. We are after "usual best function" here, and neither supramaximal nor insufficient efforts at performance. The FS equivalents are advisory and not prescriptive for these and higher steps.

EDSS Step 5.0 requires ambulation for about 200 meters without aid or rest. Disability is severe enough to impair full daily activities, eg, to work a full day without special provisions. Usual FS equivalents are one grade 5 alone, others 0 or 1, or combinations of lesser grades that will usually exceed those specified for EDSS step 4.0.

EDSS Step 5.5 requires ambulation for some 100 meters without aid or rest. Other criteria are inability to work part-time (about ½ day) without special provisions. Usual FS equivalents are as in step 5.0. Note the arbitrary distances for walking ability.

EDSS Step 6.0 requires assistance to walk about 100 meters. This may mean resting, the use of unilateral aids (cane, crutch, or brace) at most times, or the intermittent use of bilateral aids. The assistance of another person also counts as "with aid." The

Table 6. Percentage frequency distribution of Functional System (FS) grades according to DSS steps, IV: DSS 7-9*

DSS 7-9 FS	FS grades 0	1	2	3	4	5	6	100% = (N)
	(percentages)							
P	0.7	1.3	1.0	3.0	40.6	45.9	7.5	(305)
Cll'	1.0	—	4.0	23.2	49.5	22.2	NA	(99)
BS	17.9	12.1	16.8	32.8	15.7	4.6	NA	(280)
S‡	28.1	4.6	33.6	29.9	---------3.7---------		NA	(217)
BB‡	20.3	3.6	12.7	27.9	7.6	27.9	NA	(276)
V§	54.1	4.4	8.1	17.0	----------16.3----------			(135)
Ch	67.9	17.4	---------5.8---------		4.7	4.7	NA	(172)
O	57.9	42.1	NA	NA	NA	NA	NA	(328)

* Data from some 2,000 exams in 20 years among 527 MS males, Army WWII series.
' Excludes those with Pyramidal grade 3+.
‡ 1961 scales.
§ VA Hospital series (N = 392).
— No cases.
NA Not applicable; step(s) not on the scale.

primary measure for this step is the ability to walk with help for about 100 meters. Usual FS equivalents are combinations with more than two FS grade 3+.

EDSS Step 6.5 requires assistance to walk about 20 meters without resting by means of aids (canes, crutches, braces, or people), which are generally bilateral and generally constantly necessary. Usual FS equivalents are as in 6.0—combinations with more than two FS grade 3+. A person who cannot walk 20 meters is functionally almost nonambulatory and should be considered close to DSS 7.

DSS Steps 7-9. These are the severely involved patients who are almost invariably limited to wheelchair or bed. Table 6 demonstrates the marked shift to the right for FS grade involvement, particularly those functions having to do with ambulation. This behavior of groups of MS patients lends validity to a scoring system that stresses ambulation in the higher ranges; only in the most severe will the loss of upper limb and head functions be added. The ratio of the steps here was about 1.4:1:1.

The original definition of DSS step 7 was "restricted to wheelchair (able to wheel self and enter and leave chair alone).... It does not include the patient who is tied in the chair and perambulated."[1] Conversely, ability to walk short distances is not sufficient to qualify for step 6. The arbitrary limit for "short distances" is taken here as about 5 meters. This provides some leeway between EDSS step 6.5 (20 meters) and 7.0 (5 meters). As with the other grades, assignment is to that closest to his performance.

EDSS Step 7.0 defines essential restriction to wheelchair with inability to walk beyond about 5 meters even with aid. Patients can transfer alone (with mechanical aids if needed) and wheel the standard wheelchair; are able to be up and about in the chair some 12 hours a day; with the chair, are not housebound and may even be employed. Usual FS equivalents are combinations with more than one FS grade 4+; rarely, Pyramidal grade 5 alone.

EDSS Step 7.5 describes inability to take more than a few steps and, essentially, restriction to wheelchair. With or without aid, these patients can transfer. They can wheel themselves, but cannot carry on in standard wheelchair a full day. They may require motorized wheelchair for ability to be up and about in the chair. Usual equivalents are combinations with more than one grade 4+.

EDSS Step 8.0. The original DSS 8 definition was "restricted to bed but with effective use of the arms ...; he can usually feed himself and perform part of his toilet."[1] In our setting, it has been standard procedure to get bed patients into chairs as much as possible, so that the horizontal posture was not a requirement for "bed patient." This (to me) obvious point has led to some confusion as to requirements for DSS 8.

EDSS Step 8.0 is defined as bed patients who may be in chair or (passively) in wheelchair for much of the day, and it is so specified in appendix B. Primarily, though, they retain many self-care functions and generally have effective use of the arms. Usual FS equivalents are combinations, generally grade 4+ in several systems.

EDSS Step 8.5 are the bed patients who in daytime generally cannot tolerate prolonged periods in chair and are more often in bed, unless tied in the chair. Primarily, they still have some effective use of one or both arms and can perform some self-care functions, but less than for step 8.0. Usual FS equivalents are as in step 8.0.

EDSS Step 9.0 are the "helpless bed patients" who, however, can communicate and eat. They cannot perform self-care functions (such as feeding). Usual FS

Table 7. DSS: Percentage frequency distribution in two series of MS patients at examination

DSS	Series 1*	Series 2†
0	0.3	5.4
1	0.6	3.7
2	6.9	8.3
3	12.6	19.8
4	16.0	19.8
5	16.0	15.7
6	13.1	9.1
7	18.3	7.7
8	12.3	4.8
9	4.0	5.6
10	NA	NA
Total	100.1	99.9
N	(350)	(1,665)

* VA Hospital series, admission status (data are percentages).
† Army WWII series, overview for some 20 years (data are percentages).

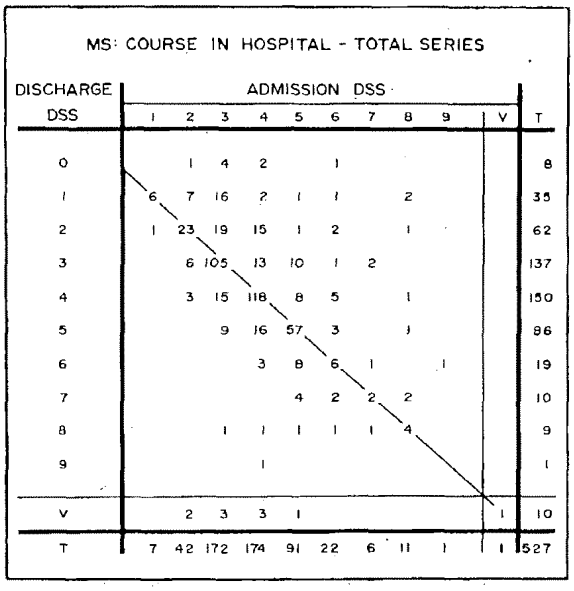

Figure. Grid correlate of DSS at admission to and discharge from hospital for an early bout of MS; Army WW II series.[7] Numbers along the main diagonal (0, 0 . . . 9, 9) indicate no change in DSS between admission and discharge; those above the diagonal improved and those below worsened, by the number of steps off diagonal for each locus.

equivalents are combinations, mostly grade 4+.

EDSS Step 9.5 defines the totally helpless bed patients who cannot communicate effectively, eat, or swallow. Usual FS equivalents are combinations, almost all grade 4+.

EDSS Step 10 is death due to MS. This may be an acute death due to "brainstem" involvement or to respiratory failure,[9] or death consequent to the chronic bedridden state with terminal pneumonia, sepsis, uremia, cardiorespiratory failure. It excludes intercurrent causes of death. Antemortem, the patient will ordinarily be DSS 9, sometimes 8.

Discussion. The expanded DSS should answer the needs of those who felt constrained by too few steps in the original scale. The reason each step had to be divided, rather than only a few steps, may be seen in table 7, which shows the distribution of DSS scores in two series of MS patients. In our hands, at least, the distribution was reasonably Gaussian, and no single step stood out as markedly discrepant. With this evidence, the DSS could in fact be treated as a true numerical scale, with means and standard deviations, rather than the ordinal (rank) scale that is its basic structure. This would imply that DSS 6 is twice as "bad" as DSS 3.

In several studies, a clearly bimodal distribution of DSS scores was found. Comparing the individual FS scores with DSS in many of these (published and unpublished) suggests that the DSS scores below 6 had been assigned with little regard to FS grades. This should be obviated if the new EDSS is used—or even if the old DSS were retained, but with the FS equivalents given here. The sum of the two EDSS steps of the same number, eg, 2.0 + 2.5, would be identical with the old DSS number, ie 2. In one unpublished study, one DSS step was dramatically higher than all others. I suspect this was miscoding of contiguous steps, since nowhere else have I seen this.

The thesis that the DSS is a true numerical, equal-interval scale, though, is irrelevant to what I believe to be the proper handling of the scale as an index of neurologic change with time. To me, the Gaussian appearance is important principally in deciding that no one step is superfluous, and that no one step is really two or more steps on the continuum from normal to maximal disease. This appearance of a normal distribution is the basic reason for the EDSS as presented, with each prior step divided in half.

As to my preferred way of handling DSS scores over time, it remains the same as previously. Improvement or worsening for each patient was defined as a gain or loss of at least one step on the DSS. This should not happen unless at least one FS changed by an equivalent degree in the same direction. The plotting of cases at two intervals would then be most easily accomplished by a grid correlate of DSS scores at time 1 versus DSS scores at time 2. The numbers moving off the major diagonal of no change provide the numbers improving or worsening by one, two, three or more steps (figure). Then the proportions better-same-worse could be compared between two regimens if this were a therapeutic trial.

With the EDSS, a gain/loss of 0.5 steps will be defined as better/worse, but again, greater changes can be recorded. I cannot assert that each EDSS gain of 0.5

should be accompanied by a change in FS of at least one grade, but I would be suspicious of the DSS change if this were not evident.

In other words, despite the Gaussian configuration of the DSS, I still prefer to treat it as an ordinal scale. For the FS, the only proper assessment is to consider each System individually, to plot "in" versus "out" as a grid correlate as with the DSS, and then to look at proportions changing *in simile modo*. Further, the FS scores are not additive, and each system can be compared only with itself. One obvious reason is that as Pyramidal worsens, Cerebellar will "improve," since patients cannot be ataxic if they cannot move. The lack of additivity in these systems was the underlying reason for the DSS. Also, I believe that mean FS scores are difficult to defend, even when speaking only to the individual systems. The distributions for most of them are clearly non-Gaussian (tables 3 through 6), and they also have differing configurations one versus another.

In the introduction, another impetus behind the paper was mentioned. The International Federation of Multiple Sclerosis Societies (IFMSS) is trying to establish a Uniform Minimum Record of Disability, which would be internationally acceptable as a way to characterize MS patients.[10] Three separate scales were desired: one rating scheme to record the neurologic signs, one to record the physical disabilities or impairments, and one to record the societal impact of the disease. With differing labels, this follows the schema recommended by the World Health Organization to classify the consequences of disease according to "impairments" (neurologic abnormalities), "disabilities," and "handicaps."[11]

At a meeting in Stockholm[12] it was thought that, for what by WHO was called (neurologic) "impairment," the rating scheme presented here—the DSS plus FS—was the most likely to meet with, if not universal acceptance, at least minimal opposition when compared with other proposals. The wide use of this method was documented.[12] For the physical impairments or "disabilities" resulting from the disease, an Incapacity Scale was devised—a term chosen deliberately because it had not yet been appropriated by any other scheme.[13] The societal impact (WHO: "handicaps") was assayed by what was then called a Socio-Economic Scale.[14] Both the latter scales have been undergoing revisions, the economic one most drastically. IFMSS is continuing these efforts to establish and test a common tripartite scheme that would be suitable for all centers.

Appendix A. Functional Systems.

Pyramidal Functions

0. Normal.
1. Abnormal signs without disability.
2. Minimal disability.
3. Mild or moderate paraparesis or hemiparesis; severe monoparesis.
4. Marked paraparesis or hemiparesis; moderate quadriparesis; or monoplegia.
5. Paraplegia, hemiplegia, or marked quadriparesis.
6. Quadriplegia.

V. Unknown.

Cerebellar Functions

0. Normal.
1. Abnormal signs without disability.
2. Mild ataxia.
3. Moderate truncal or limb ataxia.
4. Severe ataxia, all limbs.
5. Unable to perform coordinated movements due to ataxia.

V. Unknown.

X. Is used throughout after each number when weakness (grade 3 or more on pyramidal) interferes with testing.

Brain Stem Functions

0. Normal.
1. Signs only.
2. Moderate nystagmus or other mild disability.
3. Severe nystagmus, marked extraocular weakness, or moderate disability of other cranial nerves.
4. Marked dysarthria or other marked disability.
5. Inability to swallow or speak.

V. Unknown.

Sensory Functions (revised 1982)

0. Normal.
1. Vibration or figure-writing decrease only, in one or two limbs.
2. Mild decrease in touch or pain or position sense, and/or moderate decrease in vibration in one or two limbs; or vibratory (c/s figure writing) decrease alone in three or four limbs.
3. Moderate decrease in touch or pain or position sense, and/or essentially lost vibration in one or two limbs; or mild decrease in touch or pain and/or moderate decrease in all proprioceptive tests in three or four limbs.
4. Marked decrease in touch or pain or loss of proprioception, alone or combined, in one or two limbs; or moderate decrease in touch or pain and/or severe proprioceptive decrease in more than two limbs.
5. Loss (essentially) of sensation in one or two limbs; or moderate decrease in touch or pain and/or loss of proprioception for most of the body below the head.
6. Sensation essentially lost below the head.

V. Unknown.

Bowel and Bladder Functions (revised 1982)

0. Normal.
1. Mild urinary hesitancy, urgency, or retention.
2. Moderate hesitancy, urgency, retention of bowel or bladder, or rare urinary incontinence.

3. Frequent urinary incontinence.
4. In need of almost constant catheterization.
5. Loss of bladder function.
6. Loss of bowel and bladder function.

V. Unknown.

Visual (or Optic) Functions

0. Normal.
1. Scotoma with visual acuity (corrected) better than 20/30.
2. Worse eye with scotoma with maximal visual acuity (corrected) of 20/30 to 20/59.
3. Worse eye with large scotoma, or moderate decrease in fields, but with maximal visual acuity (corrected) of 20/60 to 20/99.
4. Worse eye with marked decrease of fields and maximal visual acuity (corrected) of 20/100 to 20/200; grade 3 plus maximal acuity of better eye of 20/60 or less.
5. Worse eye with maximal visual acuity (corrected) less than 20/200; grade 4 plus maximal acuity of better eye of 20/60 or less.
6. Grade 5 plus maximal visual acuity of better eye of 20/60 or less.

V. Unknown.

X. Is added to grades 0 to 6 for presence of temporal pallor.

Cerebral (or Mental) Functions

0. Normal.
1. Mood alteration only (Does not affect DSS score).
2. Mild decrease in mentation.
3. Moderate decrease in mentation.
4. Marked decrease in mentation (chronic brain syndrome—moderate).
5. Dementia or chronic brain syndrome—severe or incompetent.

V. Unknown.

Other Functions.

0. None.
1. Any other neurologic findings attributed to MS (specify).

V. Unknown.

Appendix B. Expanded Disability Status Scale (EDSS)

0 = Normal neurologic exam (all grade 0 in Functional Systems [FS]; Cerebral grade 1 acceptable).

1.0 = No disability, minimal signs in one FS (ie, grade 1 excluding Cerebral grade 1).

1.5 = No disability minimal signs in more than one FS (more than one grade 1 excluding Cerebral grade 1).

2.0 = Minimal disability in one FS (one FS grade 2, others 0 or 1).

2.5 = Minimal disability in two FS (two FS grade 2, others 0 or 1).

3.0 = Moderate disability in one FS (one FS grade 3, others 0 or 1), or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) though fully ambulatory.

3.5 = Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3; or five FS grade 2 (others 0 or 1).

4.0 = Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest some 500 meters.

4.5 = Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability, usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 300 meters.

5.0 = Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (eg, to work full day without special provisions). (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0.)

5.5 = Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities. (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding those for step 4.0.)

6.0 = Intermittent or unilateral constant assistance (cane, crutch, or brace) required to walk about 100 meters with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+.)

6.5 = Constant bilateral assistance (canes, crutches, or braces) required to walk about 20 meters without resting. (Usual FS equivalents are combinations with more than two FS grade 3+.)

7.0 = Unable to walk beyond about 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in w/c some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely, pyramidal grade 5 alone.)

7.5 = Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair. (Usual FS equivalents are combinations with more than one FS grade 4+.)

8.0 = Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade 4+ in several systems.)

8.5 = Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally 4+ in several systems.)

9.0 = Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4+.)

9.5 = Totally helpless bed patient; unable to communicate effectively or eat/swallow. (Usual FS equivalents are combinations, almost all grade 4+.)

10. = Death due to MS.

References

1. Kurtzke JF. A new scale for evaluating disability in multiple sclerosis. Neurology (Minneap) 1955;5:580-3.
2. Kurtzke JF, Berlin L. The effects of isoniazid on patients with multiple sclerosis: preliminary report. AM Rev Tuberc 1954;70:577-92.
3. Veterans Administration Multiple Sclerosis Study Group. Isoniazid in treatment of multiple sclerosis. Report on Veterans Administration cooperative study. JAMA 1957;163:168-72.
4. Kurtzke JF, Berlin L. Isoniazid in treatment of multiple sclerosis. JAMA 1957;163:172-4.
5. Kurtzke JF. On the evaluation of disability in multiple sclerosis. Neurology (Minneap) 1961;11:686-94.
6. Kurtzke JF. Further notes on disability evaluation in multiple sclerosis, with scale modifications. Neurology (Minneap) 1965;15:654-61.
7. Kurtzke JF, Beebe GW, Nagler B, Auth TL, Kurland LT, Nefzger MD. Studies on the natural history of multiple sclerosis. 6. Clinical and laboratory findings at first diagnosis. Acta Neurol Scand 1972;48:19-46.
8. Kurtzke JF. Neurologic impairment in multiple sclerosis and the Disability Status Scale. Acta Neurol Scand 1970;46:493-512.
9. Guthrie TC, Kurtzke JF, Berlin L. Acute respiratory failure in multiple sclerosis and its management. Ann Intern Med 1952;37:1197-203.
10. Slater RJ. International Symposium on MS services: introductory remarks. Functional disability ratings: medical, social and economic implications. Acta Neurol Scand (Suppl 87) 1981;64:3-4.
11. Wood PHN. Appreciating the consequences of disease: the international classification of impairments, disabilities, and handicaps. WHO Chron 1980;34:376-80.
12. Slater RJ, Fog T, Bergmann L. International Federation of Multiple Sclerosis Societies. A Festschrift for Professor Tore Broman. Symposium on multiple sclerosis services. Functional disability ratings: medical, social and economic implications. Stockholm, Sweden, August 31-September 1, 1980. Acta Neurol Scand (Suppl 87) 1981;64:1-138.
13. Kurtzke JF. A proposal for uniform minimal record of disability in multiple sclerosis. Acta Neurol Scand (Suppl 87) 1981;64:110-29.
14. Mellerup E, Fog T, Raun N, et al. The socio-economic scale. Acta Neurol Scand (Suppl 87) 1981;64:130-8.

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Cladribine

A Review of its Use in Multiple Sclerosis

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Contents

Summary

Cladribine is a deaminase-resistant deoxyadenosine analogue that selectively reduces lymphocyte counts. The drug is an effective therapy for selected haematological malignancies and is being tested in patients with multiple sclerosis (MS),

in whom the antilymphocytic effects of the drug may reduce the autoimmune destruction of myelin.

With activity against resting and dividing cells that express high deoxycytidine kinase activity, cladribine causes prolonged, profound suppression of lymphocyte counts. Subcutaneous cladribine is 100% bioavailable and has no local tissue toxicity. Dosages used in clinical trials in patients with MS are in the range of 0.05 to 0.07 mg/kg/day subcutaneously for 5 days each month for 2 to 6 months.

Temporary improvement or no change in neurological functioning and improvements in CNS lesions detected by gadolinium-enhanced magnetic resonance imaging (MRI) have been seen after cladribine use in patients with chronic progressive (CPMS) and relapsing-remitting (RRMS) forms of MS. In a randomised double-blind study of 24 pairs of patients, improvement or stabilisation of CPMS for ≥2 years was observed in cladribine-treated patients, whereas the disease progressed in placebo recipients. Another study of 159 patients found no progression in either the treated or placebo control group. In both studies, marked improvements were seen in gadolinium-enhanced CNS lesions. Cladribine-associated improvements in neurological functioning were also seen in some patients with RRMS in one study, which also noted a reduction in the frequency and severity of relapses. In this and a separate RRMS study, cladribine resulted in the regression of CNS lesions on MRI.

Bone marrow suppression is the main dose-related toxicity; in patients with MS, use of low total cladribine dosages appears to limit myelosuppression. Although thrombocytopenia is of concern with higher-dose regimens (i.e. 2.8 mg/kg total dose) in patients with MS, granulocyte counts and haemoglobin levels appear to be largely unaltered. Cladribine treatment is also associated with culture-negative fever and a risk of infections in patients with haematological malignancies.

Conclusions: Further study of cladribine is needed to confirm present results in wider numbers of patients treated or followed up for longer durations, define optimum treatment and retreatment schedules for the drug and compare it with other agents. Nonetheless, cladribine therapy appears to have the potential to slow the progression of MS, reduce CNS lesions in patients with either the chronic progressive or relapsing-remitting forms of the disease and improve neurological functioning in some of these patients.

1. Rationale for the Use of Cladribine in Multiple Sclerosis

Multiple sclerosis (MS) is characterised by clinical signs and symptoms of CNS demyelination including optic neuritis, diplopia, muscle weakness, spasticity and eventual loss of ambulatory function. The disease may have relapsing and remitting stages (RRMS) or can be chronic and progressive (CPMS).[1,2] However, progressive disease may occur without a relapsing-remitting stage (primary progressive) or may follow a relapsing-remitting stage (secondary progressive).[3] The disease is highly variable in its course, and although it caus considerable disability, it does not greatly redu life expectancy, except in patients with severe di ability.[4]

A diagnosis of MS must be based on symptor characteristic of at least 2 CNS lesions. Tests th assist in diagnosis and evaluation of the progre sion of MS include examination of the CSF for t presence of oligoclonal bands of IgG and magnet resonance imaging (MRI) for CNS lesions.[5] Ne rological impairment is often assessed using t Kurtzke extended disability status scale (EDSS although the Scripps neurological rating sca

(SNRS) and other similar tests have also been used.[2] For a more detailed description of the functions of these tests, see section 3 and the review by Waubant and Goodkin.[2]

Cladribine (2-chloro-2′-deoxyadenosine) is an adenosine deaminase–resistant analogue of deoxyadenosine. After phosphorylation to cladribine triphosphate within cells by the enzyme deoxycytidine kinase (dCK), it is incorporated into DNA, where it takes the place of adenosine triphosphate and effectively halts cell replication.[6] Phosphorylated cladribine can be dephosphorylated (and therefore inactivated) by 5′-nucleotidase (5-NT). Of various cells of the body, lymphocytes are most subject to the effects of cladribine because they have a higher ratio of dCK to 5-NT than other cells. This and other mechanisms confer on cladribine specific antilymphocytic effects that are of clinical utility in the treatment of haematological malignancies (reviewed by Bryson & Sorkin[6]).

Although MS is thought to be a lymphocyte-dependent autoimmune disease, the specific antigens and triggering agents involved in the disease are unknown, so treatments are nonspecific.[7] The rationale for the use of cladribine in MS is that autoantigen-specific T lymphocytes are thought to be activated peripherally before migrating to the CNS, where they mediate damage to myelin.[8] This suggests that a cladribine-induced reduction in the number of lymphocytes may help to slow progression of the disease. The efficacy of cladribine in the treatment of MS has been studied and the evidence relating to its potential use in this disease is reviewed here.

2. Pharmacology

2.1 Pharmacodynamics

2.1.1 Pharmacodynamic Effects and Mechanism of Action

The major pharmacodynamic effects of cladribine are on blood cells and blood progenitor cells, which express high levels of dCK. dCK levels, which correlate with the degree of cladribine phosphorylation, are high in normal leucocytes (120ng dCK/mg protein) and low in other tissues such as stomach mucosa (6 ng/mg).[9] Cladribine also markedly and dose-relatedly inhibits lymphocyte colony-forming and myeloid progenitor cells from normal human peripheral blood and bone marrow *in vitro*.[10] Bone marrow suppression is a dose-limiting adverse effect of cladribine (see section 4).

Cladribine is active against both resting and dividing cells; therefore, at least 2 mechanisms are thought to be involved in its activity.

In dividing cells, cladribine is believed to be incorporated into DNA in its triphosphate form after phosphorylation by dCK. Cladribine appears in much higher concentrations within blood cells than in blood plasma (see section 2.2.1) and its cytotoxic effects on leukaemic cells correlate with the efficiency of its transport across the cell membrane.[11] Within cells, it is phosphorylated by dCK and dephosphorylated by 5-NT; thus, it is not surprising that hairy cell leukaemia (HCL) and chronic lymphocytic leukaemia (CLL) cells from patients with leukaemia who responded to cladribine exhibited higher dCK ($p < 0.01$) and lower 5-NT ($p < 0.05$) levels than those from non-responders.[12] Cladribine is resistant to adenosine deaminase, so phosphorylated forms of the drug accumulate within cells; 80% of a radiolabelled dose was identified as cladribine monophosphate and 10% as the triphosphate in human tonsillar lymphocytes *in vitro*.[13] Incorporation of cladribine triphosphate into the DNA of dividing cells appears to arrest cell division.[6]

In resting cells, cladribine is believed to induce apoptosis, or programmed cell death. DNA fragmentation is known to occur in a dose-related manner when cells from patients with CLL are exposed to cladribine *in vitro*.[14] Cladribine also appears to induce expression of the p53 protein and its downstream target WAF1/CIP1 protein, which have been implicated in the apoptosis response to DNA damage.[15] Apoptosis has also been measured in peripheral blood cells from 3 patients with HCL before and after intravenous cladribine (0.09 mg/kg/day infused for 7 days),[16] rising from 2 to 3.4% at baseline to 20 to 32% after 5 to 14 days.

Table I. Effects of cladribine (CdA) on blood cell counts in patients with multiple sclerosis of the chronic progressive (CPMS) o relapsing-remitting (RRMS) forms

Parameter	Scripps IV CPMS (n = 29; 30mo observation)[19-21]	Scripps SC RRMS (n = 7; 10mo observation)[20]	Polish SC or PO RRMS (n = 11, 18mo observation)[22]	Polish SC RRMS (n = 90; ≈18mo observation)[23]
Dosage	CdA 0.087-0.1 mg/kg/day IV × 7 days q1mo × 4mo	CdA 0.07 mg/kg/day SC × 5 days q1mo × 6mo	CdA 5 mg/day SC or 10 mg/day PO × 5 days q1mo × 6mo, plus 1 or 2 additional courses at 3mo or 6mo intervals in some patients	CdA 5mg od SC × 5 days q1mo × 6mo; then 5mg od 5 day 3mo later
Lymphocytes	Prolonged profound lymphopenia (especially of CD4+ cells) affecting both T (CD3+) and B (CD19+) cells; decreased CD4+/CD8+ ratio	Prolonged profound lymphopenia	Prolonged and profound decrease (from ≈2.5 × 10^9 to 1 × 10^9 cells/L), no correlation between decrease at 6mo and CdA dosage	Decrease to one-third of original counts
Monocytes	Acute transient monocytopenia	?	?	?
Granulocytes	Modest decrease	?	Little mean change (small decrease at 18mo)	'Not reduced significantly'
Haemoglobin	Modest decrease	Modest decrease	No change	?
Cell size	Prolonged macrocytosis	No macrocytosis	Slight macrocytosis in 'some patients', change in average MCV NS except at 18mo	'Some macrocytosis'
Platelets	Sharp decrease in counts for 6mo, nadir at 8mo, counts were <100 × 10^9 cells/L in 7 of 29 patients (24%)	Variable platelet counts, no major change from baseline at 10mo	Decrease, but none <100 × 10^9 cells/L	'Mild' thrombocytopenia 'of no clinical significance'

Abbreviations and symbol: IV = intravenous; MCV = mean corpuscular volume; NS = not statistically significant; od = once daily; PO = or q1mo = every month; SC = subcutaneous; ? = not reported.

Additional *in vitro* effects that may contribute to the mechanisms of action of cladribine include modification of the activity of DNA polymerase[17,18] and ribonucleotide reductase[18] and induction of dCK activity.[13] For further discussion of the mechanism of activity of cladribine, see the review by Bryson and Sorkin.[6]

2.1.2 Effects in Patients with Multiple Sclerosis

Cladribine has a clear profile of effects on lymphocytes and other blood cell counts in patients with MS (table I).

A marked and sustained reduction in lymphocyte counts appears to occur regardless of the total cladribine dose, type of MS or route of drug administration. In 4 trials (1 in patients with CPMS and 3 in patients with RRMS),[20-23] subcutaneous, oral or intravenous cladribine use was associated with reductions in lymphocyte counts to ≈1 × 10^9 cells/L or to at least one-half and up to one-third of baseline counts, and these reductions lasted throughout the 10- to 30-month observation periods.

Effects of cladribine on other haematologic parameters (e.g. platelet counts, cell size) appear be related to the total dose and/or exposure perio They are greatest in patients with CPMS who r ceived total doses of 2.8 mg/kg as 7-day intr venous courses (table I).[20-22] The lesser effec seen in studies of patients with RRMS are n thought to be related to the disease type or the rou of administration, but to the lower dosages (0.(mg/kg or 5mg once daily subcutaneously or 10n once daily orally) and the shorter courses (5 vs days) adopted in the RRMS trials. Indeed, althou thrombocytopenia and prolonged macrocytosis o curred with the higher total dose (2.8 mg/kg) in t patients with CPMS, only modest effects on plat let counts and cell size were observed in patien with RRMS receiving lower total doses (2

mg/kg) or lower daily dosages for shorter courses (5mg subcutaneously or 10mg orally for 5 days).

Little or no effect was seen on mean granulocyte counts or mean haemoglobin levels in these studies (table I). Monocytopenia was reported in the intravenous study, but was not discussed in reports of the other trials. For a discussion of other adverse effects of cladribine, see section 4.

2.2 Pharmacokinetics

As yet, no pharmacokinetic studies of cladribine have been conducted in patients with MS, but the kinetics of the drug are well studied in patients with haematological malignancies and solid tumours. Most of the pharmacokinetic research has been conducted in Sweden, and the kinetics of cladribine have been reviewed recently by Liliemark.[24] An overview of cladribine pharmacokinetics based on the review of Liliemark is presented in table II.

The only commercially available form of cladribine is an intravenous injectable solution (1 mg/ml in normal sodium chloride and phosphate buffer),[26,27] but the kinetics of the drug have been tested after intravenous, oral, subcutaneous and rectal administration. Other solutions have been prepared for subcutaneous administration (buffered 2.5 mg/ml at pH 7.4) or oral use (isotonic 1 mg/ml),[22] and the drug has been formulated into enteric-coated capsules for oral testing.[28] Although the kinetics of the drug after oral and rectal administration have been examined in patients with malignancies[24] and the oral drug has been tested in clinical trials of patients with MS,[23] bioavailability of these 2 forms is low (≤51% oral; ≈20% rectal) and variable, so their use in patients with MS is not generally recommended (see section 5). Also, although originally used as a continuous 24-hour intravenous infusion, intravenous cladribine is now usually administered to patients with MS as 2-hour infusions once daily or as subcutaneous injections; these regimens/routes appear to result in pharmacokinetics and efficacy similar to those seen with use of the intravenous route.[24]

Table II. Overview of cladribine pharmacokinetic parameters in adult patients with haematological malignancies (data from the review by Liliemark[24] unless otherwise noted)

Parameter	Route		
	IV	SC	PO
F (%)		100	37-51
C_{max} (nmol/L)	142 after 2h 0.12 mg/kg infusion	268 after 0.12 mg/kg	165 after 0.24 mg/kg
$t_{1/2\alpha}$ (min)	8-11.9		
$t_{1/2\beta}$ (h)	0.7-1.5		
$t_{1/2\gamma}$ (h)	5.7-13.4		
V_{ss} (L/m²)	54-368		
CL (L/h/m²)	26-45		
CSF : plasma concentration ratio	0.25		
PB (%)[25]	Patients with haematological malignancies: 25 Healthy volunteers: 21.1 Human serum albumin *in vitro*: 24.3		

Abbreviations: CL = clearance; C_{max} = peak plasma concentration; F = bioavailability; IV = intravenous; PB = plasma protein binding; PO = oral; SC = subcutaneous; $t_{1/2\alpha}$ = distribution half-life; $t_{1/2\beta}$ = elimination half-life; $t_{1/2\gamma}$ = terminal elimination half-life; V_{ss} = volume of distribution at steady-state.

2.2.1 Absorption and Distribution

Having no local tissue toxicity and 100% bioavailability, cladribine is suitable for administration as a subcutaneous injection.[24] Variability in the area under the plasma concentration-time curve (AUC) is high between individuals, but is similar after oral and intravenous administration (coefficient of variation 38 *vs* 36%).[24] Oral cladribine has low (37 to 51%) bioavailability. Food slowed and reduced oral absorption of cladribine. Although cladribine is unstable at low pH, oral bioavailability was not substantially increased by raising stomach pH with omeprazole.[24]

Cladribine has a short distribution half-life of 8 to 11.9 hours and is widely distributed in the body cells, with a steady-state volume of distribution of up to 368 L/m². Concentrations of the drug and its nucleotides within cells are several hundred times higher than concentrations of the parent drug in plasma, but cannot be predicted by plasma concentrations. Cladribine and its metabolites are also retained in leukaemic cells, with a half-life of up to 30 hours, making the drug suitable for intermittent

rather than continuous administration. Cladribine also distributes to the CSF, and although concentrations are lower there than in the plasma, the time course of CSF pharmacokinetics is roughly similar to that in plasma.[24]

2.2.2 Metabolism and Excretion

Much of an orally administered dose may be deglycosylated to chloroadenine before absorption, resulting in the lower systemic availability of oral than intravenous cladribine.[29] Chloroadenine is a major inactive catabolite of cladribine; its AUC is similar to that of cladribine after intravenous administration of 5 mg/m^2 (530 *vs* 508 nmol/L · h), but it has a 4.4-fold greater AUC than cladribine after oral administration (1863 *vs* 423 nmol/L · h).[29]

Renal clearance is 51% of total systemic clearance of cladribine.[24] 21 to 32% of an intravenously administered cladribine dose was excreted in the urine within 24 hours as unchanged drug[24] and <10% was excreted as chloroadenine.[24,29]

There are no data on the pharmacokinetics of cladribine in patients with impaired renal or hepatic function.

3. Therapeutic Potential in Multiple Sclerosis

The results of 2 pilot studies suggested that patients with CPMS[30,31] and RRMS[32,33] might benefit from cladribine treatment and that benefits may be due to immunological mechanisms. In 4 patients with clinically or laboratory-supported definite CPMS of ≥2 years' duration, cladribine 0.087 mg/kg/day intravenously for 7 days once a month for 6 months (total cumulative dose 3.7 mg/kg) resulted in reported improvements in clinical manifestations of the disease (assessed using the SNRS) and the disappearance of oligoclonal bands from the CSF.[30,31] In 10 patients with RRMS and 'seemingly irreversible clinical deficits' on the EDSS, cladribine 5 mg/day subcutaneously or 10 mg/day orally was administered for 5 days once monthly for 6 months followed by 2 additional courses at 3-month intervals.[32,33] The number of relapses was unchanged in 3 patients and reduced markedly in the other 7, and EDSS scores decreased from a mean of 4.3 at baseline to 2.4 after 15 months' observation before gradually returning to baseline by 24 months.[33]

None of the published reports of studies examined in this review differentiated between patients with primary progressive or secondary progressive forms of CPMS. To the extent that it is known, this information should be provided in reports of future clinical trials.

Additional and longer term trials examined different cladribine dosage schedules versus placebo in patients with confirmed CPMS or RRMS (see table III for study design details), and one retreatment study is also under way (results unavailable). Major study end-points were neurological functioning, MRI evidence of CNS lesions and clinical relapse. For effects of cladribine on haematologic end-points, see section 2.1.2.

The EDSS and SNRS are semiquantitative scales that were used to test neurological functioning. In general, higher SNRS and lower EDSS scores indicate better functioning. EDSS and SNRS scores may not correlate in individual patients.[39] The SNRS tends to show more gradual changes in disease course, and in a sample of CPMS patients with CPMS it was nearly normally distributed about a mean of ≈70. The EDSS shows more abrupt changes, and, in the same sample of patients, exhibited bimodal distribution with modes of 3.5 and 6 to 6.5.[39] MRI techniques can determine the extent of MS lesions in the CNS and the frequency of new disease activity, both of which assist prediction of the clinical course of the disease.[40] In particular, gadolinium-enhanced MRI, which was used in studies examined in this review, may be up to 10 times more sensitive as a marker of disease activity than clinical data.[40] In clinical trials, however, effects of drugs used to treat MS may be seen on MRI where no change may be seen in clinical findings.[41] Thus, once it has been used to show the effects of agents in pilot studies, MRI is best kept for use only as a secondary end-point in controlled clinical trials.

Table III. Design of cladribine (CdA) clinical trials in patients with multiple sclerosis

Trial and reference	No. of patients	Study design	Drug and dosage	Duration of study	End-points	Comments
Chronic progressive multiple sclerosis (CPMS)						
Scripps pilot[30,34]	4	nr, nb, nc	CdA 0.087 mg/kg/day IV × 7 days q1mo × 6mo	6mo	Clinical manifestations CSF IgG	
Scripps 2-year CPMS[19,20,35]	51	r, db, pc, co[a] then 6mo nb; patients in initial PL *vs* CdA groups matched for disease severity	*Initial cladribine group:* CdA 0.1 mg/kg/day IV × 7 days q1mo × 4mo in year 1 then PL in year 2 *OR* *Initial placebo:* PL in year 1 then CdA 0.1 mg/kg/day IV × 7 days q1mo × 1mo, then 0.05 mg/kg/day IV × 7 days q1mo × 2mo in year 2[a]	2.5y	EDSS SNRS MRI	6mo-20y illness duration at entry; also examined haematology (Beutler et al.,[20,21] see section 2.1.2)
Scripps CPMS retreatment[34]	?	≥sb	CdA 0.05 mg/kg/day SC × 5 days q1mo × 4mo	?	?	Study is ongoing
Multicentre North American CPMS[34,36]	159	r, pc, pg, db	CdA 0.07 mg/kg/day SC × 5 days q1mo × 2mo (total dose 0.7 mg/kg) *OR* CdA 0.07 mg/kg/day SC × 5 days q1mo × 6mo (total dose 2.1 mg/kg) *OR* PL	1y	EDSS SNRS MRI	Full final results not published
Relapsing-remitting multiple sclerosis (RRMS)						
Polish RRMS pilot[32,33]	10	nr, nb	CdA 5mg SC × 5 days q1mo × 6 courses *OR* CdA 10mg PO × 5 days q1mo × 6 courses, then 2 further courses at 3mo intervals	2y	EDSS Relapse rate MRI	Mean 1.8 relapses per year at entry; also examined haematology (Grieb et al.,[22] see section 2.1.2)
Polish double-blind RRMS[23,32,37]	90	r, db, pg	CdA 5mg SC × 5 days q1mo × 6mo; plus 1 further course 3mo later *OR* PL	30mo	EDSS Relapse rate	Study is ongoing; final results not published
Scripps 2-year RRMS[38]	50	r, db, pg	CdA 0.07 mg/kg/day SC × 5 days q1mo × 6mo *OR* PL	2y	EDSS SNRS MRI Relapse rate	

[a] Crossover took place after 1 year; patients receiving cladribine in year 2 (initial placebo group) were given a lower total dose (1.4 mg/kg) than those receiving it in year 1 (2.8 mg/kg, initial cladribine group).

Abbreviations: co = crossover; db = double-blind; EDSS = Kurtzke extended disability status scale; IV = intravenous; MRI = magnetic resonance imaging; nb = nonblind; nc = noncomparative; nr = nonrandomised; pc = placebo-controlled; pg = parallel group; PL = placebo; PO = oral; q*x*mo = every *x* months; r = randomised; SC = subcutaneous; SNRS = Scripps neurological rating scale; ≥sb = at least single-blind (details of blinding not reported).

Guidelines for the design of studies of MS therapies recommend placebo controls, randomisation, investigator blinding and controls for or consideration of the severity of disability, type and duration of disease and adequate study size and duration.[42] Investigator blinding is an important design measure, since nonblind evaluation of EDSS and other neurological function tests is a known source of bias in studies.[40] Considerations for study duration and sample size must account for the slow progression or remitting nature of the disease. Studies in patients with CPMS must be of sufficient duration to show a deterioration of function in untreated patients, and randomised studies must enrol a large enough number of patients to be able to detect a difference between treatments.[40] Detection of differences in neurological function, in particular, may be complicated by the fact that the most

widely used outcome measure, the EDSS, is not particularly sensitive to minor changes in disease severity.[40]

Published information about the design of comparative cladribine clinical trials suggests that most had appropriate study designs, despite the lack of detail about some trials (design details have been published in full only for the Scripps 2-year CPMS study).[19,20,35] Although pilot studies were nonblind, nonrandomised and of short duration, all comparative trials used randomisation, placebo control and both investigator and patient blinding. Most studies followed patients for at least 2 years and appeared to have enrolled adequate numbers of patients (although some studies are still ongoing). However, the North American multicentre study may have enrolled too few patients and/or followed patients for too short a duration (see section 6).

3.1 Effects on Neurological Function

3.1.1 Chronic Progressive Multiple Sclerosis

A clear effect of cladribine on neurological functioning was seen in the Scripps 2-year CPMS study in 48 patients matched for disease severity,[19] but no effect was observed in the 1-year multicentre North American trial that randomised 159 patients to receive cladribine or placebo.[36] For a discussion of the effects of methodological factors on the results of these studies, see section 6.

In the Scripps 2-year CPMS study, cladribine produced modest improvements in EDSS and SNRS scores that were sustained for 18 to 24 months, whereas placebo was associated with deteriorating neurological functioning. In addition, both the time-to-improvement and time-to-failure (indicated by changes of 1 EDSS or 10 SNRS points) showed statistically significant benefits of cladribine.[19]

EDSS scores in the group randomised to receive cladribine in the first year of study (initial cladribine group) improved from 4.8 at baseline to 4.4 at 13 months, with deterioration from baseline values occurring only after 18 months. In the group receiving placebo in year 1 (initial placebo group), EDSS scores increased from 4.7 at baseline to 5.6 at month 13; low-dose cladribine therapy begun in this group in month 13 produced an improvement that peaked at EDSS scores of 5.1 at month 19 before function deteriorated again.[19] Changes in EDSS from baseline in both patient groups are shown in figure 1a.

SNRS scores (fig. 1b) showed more sustained improvements with cladribine than were seen in EDSS scores in the 2-year Scripps CPMS study. A score of 69.6 at baseline increased to a peak of 75.7 at 18 months in the initial cladribine group and did not return to baseline until 30 months. However, in the initial placebo group, SNRS scores decreased from 68.3 to 62.1 at 12 months, then increased after the introduction of cladribine to 70.8 at month 19, after which they began a gradual decline.[19]

Investigators in the Scripps 2-year CPMS study also noted a less durable therapeutic effect of the better-tolerated lower total dose of 1.4 mg/kg than of the higher 2.8 mg/kg dose.[19] However, although there was only a minor 1.292 point difference in SNRS scores at the start of cladribine therapy, EDSS scores had already deteriorated by 0.7292 points when the initial placebo group began the lower cladribine dose in the second year of the study. Thus, without a direct comparison controlling for disease severity at the time of drug initiation, it is difficult to know if differences in durability of therapeutic effects are solely a sign of a dose-response relationship or may be attributable to disease factors.

It is also interesting that the improvements seen in neurological functioning in the Scripps 2-year CPMS study began to erode ≈1 year after the start of treatment, long before the CD4+ counts or CD4+ : CD8+ ratios began to increase (after ≈18 months).[19] However, in the multicentre North American study (n = 159), neither cladribine nor placebo recipients exhibited signs of progression on either EDSS or SNRS scales, and no differences were seen between the treatment groups. The lack of difference was thought to be due to unexpectedly good results in the placebo group,[36] but may be

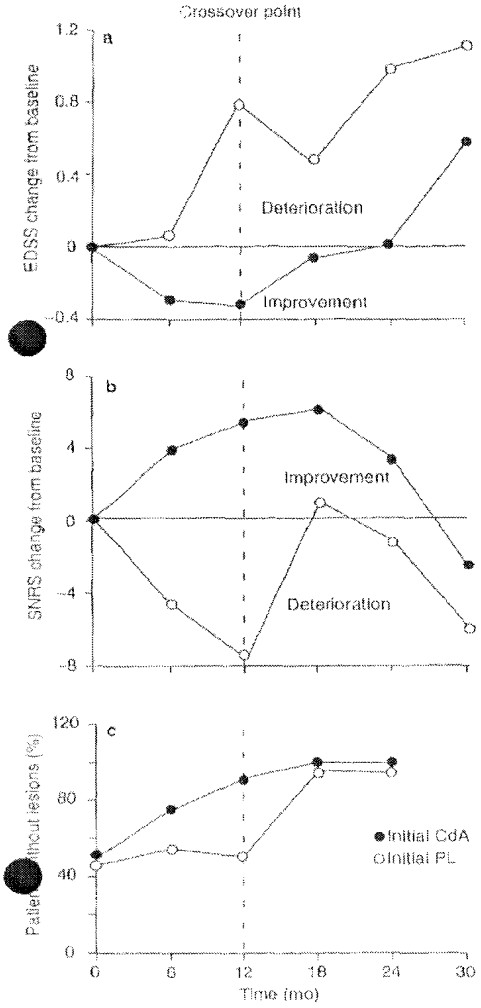

Fig. 1. Effect of cladribine (CdA) on neurological functioning and CNS lesions in patients with chronic progressive multiple sclerosis (CPMS) in the Scripps 2-year CPMS study.[19] 24 patients received intravenous CdA 0.1 mg/kg/day for 7 days for 4 monthly courses in year 1 then intravenous placebo (PL) in the second year (initial CdA group). Another 24 patients matched for disease severity received intravenous PL in the first year and in the second year received intravenous CdA 0.1 mg/kg/day for 7 days in the first month, then 0.05 mg/kg/day for 7 days once monthly for 2 more courses (initial PL group); (**a**) change in Kurtzke extended disability status scale (EDSS) from baseline; (**b**) change in Scripps neurological rating scale (SNRS) from baseline; (**c**) percentage of patients without CNS lesions detected by gadolinium-enhanced magnetic resonance imaging.

attributable to inadequate study duration or sample size (see section 6).

3.1.2 Relapsing-Remitting Multiple Sclerosis

Although neurological functioning improved in more and worsened in fewer patients with RRMS receiving cladribine than placebo, the clinical significance of this finding is doubtful. In an interim assessment of the Polish double-blind study, after 30 months of observation, 52% of 31 cladribine recipients showed improved neurological function and only 10% worsened by 1 EDSS point, *vs* 25 and 18% of 40 patients receiving placebo. Patients receiving cladribine had EDSS scores of 3.61 at entry which reduced to a statistically significant extent by 0.63 points at 30 months. In contrast, the placebo group score of 3.91 reduced nonsignificantly by 0.13 points in the same time.[32] However, changes of less than 1 point in EDSS scores are probably not clinically significant, so these changes suggest there is little real difference between groups. As yet, no published neurological function data are available from the Scripps 2-year study in patients with RRMS.

3.2 Effects on CNS Lesions and Relapse

3.2.1 Chronic Progressive Multiple Sclerosis

The number of patients with CNS lesions present on gadolinium-enhanced MRI decreased by up to 100% following cladribine therapy in the Scripps 2-year study, but did not change during placebo use (fig. 1c).

In addition, it appears that both the high (2.8 mg/kg, i.e. initial cladribine) and low (1.4 mg/kg, i.e. initial placebo) total doses of cladribine reduced the number of patients with gadolinium-enhanced lesions in the Scripps 2-year CPMS study. Furthermore, the effects of the initial high-dose regimen on MRI persisted to the end of the 30-month observation period.[19]

In the multicentre North American study, both cladribine dosages were associated with >90% suppression of gadolinium-enhanced lesions.[36] Results for placebo were not published.

Relapse was not examined in patients with CPMS.

3.2.2 Relapsing-Remitting Multiple Sclerosis

CNS lesions appeared to regress in the Scripps 2-year RRMS study, with a reduction in the number of patients with CNS lesions present on gadolinium-enhanced MRI (reported in an abstract).[38] These lesions were said to be 'virtually absent' in patients receiving cladribine, while still present in placebo recipients. In addition, the frequency and severity of relapses was reported to be significantly reduced in patients receiving cladribine. No further published details are available.

Published MRI results of the Polish double-blind study cover only 83 patients for an unspecified time from the start of treatment.[37] Although it was reported that the number of new plaques detected on MRI were twice as great in the placebo as the cladribine group (8 *vs* 4), the numbers were too small for statistical analysis.

4. Tolerability

Dose-limiting myelosuppression, culture-negative fever and infection in the weeks following treatment are the most common adverse events associated with cladribine in patients with haematological malignancies, in whom the drug has been most intensively studied.[43] Because patients are often unaware of the mostly haematological adverse events seen with cladribine,[44] careful monitoring is essential.

4.1 Haematological Events

Bone marrow suppression has been observed in patients with MS receiving high dosages of cladribine; because of this, the maximum tolerated dose is 0.1 mg/kg/day for 7 days every 28 days. Seven of 24 patients with CPMS receiving a total intravenous cladribine dose of 2.8 mg/kg in the Scripps 2-year CPMS study had thrombocytopenia ($<100 \times 10^9$ cells/L), compared with only 1 of 24 receiving a lower 1.4 mg/kg total dose.[20] The multicentre North American trial in patients with CPMS also reported that thrombocytopenia was less common at lower dosages.[36] The maximum tolerated dose in 7-day courses of cladribine every 28 days was defined in a dose-escalation study in 21 patients with nonhaematological malignan cies.[45] Myelotoxicity occurred in 1 of 7 patien receiving cladribine 0.1 mg/kg/day, compared wit 4 of 11 patients receiving 0.15 mg/kg/day and all patients receiving 0.2 mg/kg/day.[45] A total of 1 7-day courses could be given in this trial, with total dosage of ≤12.5 mg/kg. For a discussion the effects of the drug on other haematological pa rameters, see section 2.1.2.

Other adverse haematological effects occurre with cladribine, but may have been related to co comitant use of other marrow-suppres drug Two of 29 patients receiving cladribine in t Scripps intravenous trial[21,22] developed aplast anaemia and required transfusions of erythrocyt and platelets. However, 1 patient had receiv chlorambucil 950mg in the previous year and t other had received carbamazepine and phenyto before or during cladribine use. In contrast, transfusions were needed in any of 11 patients the Polish trial[22] (results on these end-points a unavailable for the Scripps RRMS trial).[20]

4.2 Fever, Neutropenia and Infection

According to the manufacturer's prescribing i formation, approximately two-thirds of patien with HCL experienced fever following cladribi administration, but only one-third of those feve were associated with documented infection.[26] F ver has not been reported as an adverse ent du ing cladribine therapy of MS, but further examin tion of this event in MS patients is needed.

Neutropenia has been clearly associated wi the cladribine AUC in patients with solid t mours,[46] which suggests that the use of low doses and shorter courses may reduce the inciden of this adverse event.

Infections were reported in patients with MS ceiving cladribine in clinical trials. Four of 48 (8 patients with CPMS in the Scripps 2-year intrav nous trial experienced herpes zoster that respond to oral aciclovir, and 1 other patient had fatal f minating hepatitis B (thought not to be related treatment).[20] One additional patient examined the Scripps group developed peritoneal cocci

aidalmycosis, which responded well to therapy (E. Beutler, personal communication). In the Polish RRMS study, several patients were reported to have 'increased frequency of upper respiratory tract infections', especially during the last 6 months of observation (1 had recurrent infections); all infections responded to standard antibacterial therapy.[22] Studies in patients with haematological malignancies suggest that opportunistic infections are most common 'in the immediate post-treatment period'.[30]

4.3 Other Events

Neurotoxicity has been reported in patients receiving cladribine at doses 4 to 9 times those usually recommended for the treatment of HCL, but this event is rare at usual HCL treatment doses[26] and has not been reported in patients with MS. No nephrotoxicity has been observed at the usual dosage used to treat HCL or MS.[27]

4.4 Long Term Follow-Up

Unpublished data on ≈35 patients treated with total cladribine doses of 2.8 mg/kg or more prior to 1992 have been gathered by the Scripps group (E. Beutler, personal communication). In 2 patients who developed persistent mild thrombocytopenia, bone marrow examination uncovered myelodysplastic changes in their marrow, including a deletion from the long arm chromosome 20 in some mitoses. Both patients were reported to be haematologically stable, requiring no therapy. Complicating the assessment of this finding is a family history of development of a myelodysplastic syndrome, occurring in the mother of 1 of these patients. Beutler reports that, because myelodysplasia is a premalignant disorder, the incidence of malignancies in all patients who received cladribine to treat MS at Scripps was analysed. In the small cohort examined, the incidence of malignancy was reported to be 'slightly under expectation' (E. Beutler, personal communication).

5. Dosage and Administration

There are no current manufacturer recommendations for the use of cladribine in patients with MS. Guidelines given here are based on clinical trials conducted in patients with MS and the manufacturer's recommendations for use of the drug in patients with haematological malignancies.

It has been recommended that all patients with MS who have received cladribine therapy and are being considered for subsequent treatment courses should satisfy the following criteria:[34]

- platelet counts of $\geq 200 \times 10^9$ cells/L *or* 150 to 200×10^9 cells/L and >50% of previous pretreatment platelet counts *or* 125 to 150×10^9 cells/L and >80% of previous pretreatment platelet counts
- absolute granulocyte counts $>10^9$ cells/L
- haemoglobin level reduced by no more than 15 g/L from previous pretreatment level *or* by no more than 30 g/L from baseline.

It is also recommended that cladribine recipients be monitored during therapy and for up to 8 weeks after treatment for the following:[26]

- haematological changes (platelet count, absolute neutrophil count, haemoglobin)
- signs of neurological toxicity (which is rare in patients with haematological malignancies and has not been reported in patients with MS)
- deterioration in renal and hepatic function
- fever, the cause of which should be investigated.

The use of cladribine should be avoided in patients with signs of active infection or haematological disorders.

The maximum recommended cladribine dosage for patients with nonhaematological malignancies was 0.1 mg/kg/day,[45] with myelosuppression being the major dose-limiting toxicity. The usual dosage for patients with active HCL is one 7-day course of 0.09 mg/kg/day given as a continuous intravenous infusion.[26] In patients with MS, the following cladribine regimens have been or continue to be tested:

- 0.05 mg/kg/day subcutaneously for 5 days each month for 4 months

- 0.07 mg/kg/day subcutaneously for 5 days each month for 2 or 6 months
- 5 mg/day subcutaneously for 5 days each month for 6 months.

Subcutaneous administration has superseded previously used intravenous infusions, being easier to use and providing similar efficacy. The use of regimens containing low total dosages is now more common in clinical studies. Oral cladribine has been used effectively in 1 published clinical trial at twice the subcutaneous daily dose.[22] However, the risk of patients absorbing the entire dose and experiencing bone marrow suppression is thought by some[20] to be too great to warrant use of cladribine by the oral route. Therefore, oral use of cladribine should be restricted to patients who may benefit from use of the drug but for whom the use of injectable dosage forms may be unacceptable. Rectal use of cladribine is not recommended (see section 2.2).

Cladribine is not recommended for women who are pregnant, breast-feeding or at risk of becoming pregnant. Caution is advised when cladribine is used in patients with renal or hepatic insufficiency, since its use in these patients has not been studied. Although there are no known drug interactions with cladribine, caution is recommended when the drug is administered before, after or with other drugs that may cause myelosuppression or immunosuppression.[26]

6. Place of Cladribine in the Management of Multiple Sclerosis

It appears that cladribine may be able to slow or temporarily reverse neurological deterioration and reduce the number of MRI-detected CNS lesions in patients with either CPMS or RRMS at high (2.8 mg/kg) and low (1.4 mg/kg) dosages. In the Scripps CPMS 2-year study, EDSS and SNRS scores improved during the first 13 months with high dose cladribine and appeared to persist beyond baseline for up to 2 years,[19] which is similar to the duration of effect of cladribine on lymphocytes in the same study.[21] It also appears that dose-limiting bone marrow suppression can be minimised through the use of cladribine dosages not exceeding 0.07 mg/kg/day. Although patients may experience opportunistic infections during cladribine therapy of MS, these tended to respond to appropriate therapy. In addition, cladribine may now be administered subcutaneously and in shorter (5 day) courses, which are more convenient than the 7-day intravenous regimens.

However, to date, details of only 2 clinical trials have been published in full; information on other trials is available mainly as abstracts. This leaves gaps in the knowledge of the effects of cladribine.

Furthermore, the results of CPMS studies are conflicting. Patients receiving placebo in the multicentre North American study showed no neurological progression, which is unusual in patients selected for having evidence of progressive disease and it has been suggested that patients in the Scripps 2-year CPMS study progressed more rapidly than is normally expected.[47] Patients receiving initial placebo in the latter trial were carefully matched with recipients of initial cladribine for similar disease severity, then each matched pair was randomised.[48] In contrast, in the shorter multicentre North American study, control for variation in disease severity was accomplished only by randomisation to cladribine or placebo groups.[3] Thus, despite enrolling more patients than the Scripps 2-year study (48 patients), the 1-year multicentre study may have had too small a patient sample (159 patients) or too short a duration to detect differences between treatment regimens in unmatched patients.[36]

In addition, CPMS studies have not clearly differentiated between patients with primary and secondary forms of the disease, which differ in their characteristics on MRI and in the speed of progression.[3] This information should be included in future study reports. Cladribine also produced clear reductions in numbers of MRI-detected lesions in most clinical trials, but improvements in neurological functioning did not always accompany these changes, a finding that supports the current view that MRI should be used only as a secondary outcome measure in MS clinical trials.[41] Thus, a

Table IV. Comparison of key features of cladribine and other immune-based therapies for multiple sclerosis

Feature	Cladribine	Interferon-β1a[50,51]	Interferon-β1b[52]	Glatiramer acetate (copolymer-1)[53]
Mechanism of action	Selective depletion of lymphocytes: ↓ CD4+/CD8+ ratio	↓ T cell surface activation markers ↑ CNS interleukin-10	Improves suppressor CD8+ T cell function ↓ T cell activation	Inhibits binding of myelin proteins to major histocompatibility complex
Route of administration	SC optimal, but IV and PO viable	IM or SC	SC	SC
Dose	5-day SC courses of 0.05-0.07 mg/kg/day or 5 mg/day at monthly intervals for 2-6 courses	30μg IM once weekly or 12-44μg 3 × weekly SC	1.6 or 8 MIU SC every other day	20mg SC once daily
Adverse events	Bone marrow suppression (dose-dependent) Fever Infection	Flu-like symptoms Headache Nausea Fever	Flu-like symptoms Injection site reactions	Injection site reactions Systemic reaction (flushing, chest tightness, dyspnoea, palpitations, anxiety)
Chronic progressive multiple sclerosis				
Results	Improved SNRS and maintained EDSS scores or produced no changes; reduced CNS lesions	No published results	No published results	Inconclusive results
Relapsing-remitting multiple sclerosis				
EDSS	Interim results suggest slowed progression in some patients	Significantly slows confirmed progression	Little effect on progression	Nonsignificant tendency to slow confirmed progression
CNS lesions	Improved	Improved	Improved	Improved
Relapse	Reduced	Reduced	Reduced	Reduced

Abbreviations and symbols: EDSS = Kurtzke extended disability status scale; IM = intramuscular; IV = intravenous; PO = oral; SC = subcutaneous; SNRS = Scripps neurological rating scale; ↓ = decreased.

though it is encouraging that MRI findings of CPMS studies indicated regression of CNS lesions during cladribine therapy, questions about study methods warrant caution in interpretation of results.

Given these considerations, longer term trials of cladribine in larger numbers of patients appear to be needed so that early results in CPMS and RRMS can be confirmed. There is still more to be learned about optimal cladribine dosages, ideal timing for retreatment and how cladribine compares with other agents used to treat MS. It may also be worth examining the potential for use of cladribine in combination with other agents used to treat MS.

With regard to comparisons, corticosteroids are a mainstay of treatment of acute exacerbations of MS. However, corticosteroids do not alter the usual course of the disease.[49] In the 1990s, the approach of considering MS as an autoimmune disease has resulted in several new therapies. A comparison between the key features of cladribine and interferons β1a and β1b and glatiramer acetate (copolymer-1) is presented in table IV.

Although all these agents act on lymphocytes, the nonselective lymphotoxic effects of cladribine may have the possible advantage of greater efficacy in patients with CPMS, but the disadvantage of dose-limiting myelotoxicity. To date, cladribine is the only one of these agents to have been shown to improve the course of CPMS (table IV); results from studies of other agents are either inconclusive or have yet to be published. For patients with RRMS, however, differences in efficacy between these agents cannot be discerned without direct comparative trials. Other factors may also play a role in the choice of therapy. Tolerability of these

agents and ability of patients or their caregivers to manage injectable therapy must be considered in management of the disease. Cost will be another consideration, but the lack of data on cost factors prevents speculation on this subject.

Thus, additional study is needed to determine whether cladribine will be useful in the management of patients with either chronic progressive or relapsing-remitting forms of multiple sclerosis. Its potential activity in this disease is based on the hypothesis that it acts by reducing the number of activated lymphocytes that can damage myelin. In patients with chronic progressive disease, it appears to result in temporary improvement or no change in neurological functioning and a reduction in CNS lesions as evinced by MRI. The few data available indicate that similar effects are also seen in patients with relapsing-remitting disease, in whom it may also reduce rates and severity of relapses. Ongoing and future studies are needed to confirm its efficacy, compare it with that of other available drugs and determine its optimal administration schedule and the optimal time for retreatment. If the preliminary results are confirmed, cladribine may come to be used to prevent or delay progression of multiple sclerosis in appropriately selected patients with chronic progressive or relapsing-remitting forms of this disease.

References

1. Hunter SF, Weinshenker BG, Carter JL, et al. Rational clinical immunotherapy for multiple sclerosis. Mayo Clin Proc 1997 Aug; 72: 765-80
2. Waubant EL, Goodkin DE. Assessing efficacy in clinical trials of treatments for multiple sclerosis: issues and controversy. CNS Drugs 1996 Dec; 6: 462-73
3. Weilbach FX, Hartung H-P. Cladribin in chronic progressive multiple sclerosis [in German]. Nervenarzt 1995 Apr; 66: 299-303
4. Weinshenker BG. The natural history of multiple sclerosis. Neurol Clin 1995 Feb; 13 (1): 119-46
5. Antel JP, Arnason BGW. Demyelinating diseases. In: Braunwald E, Isselbacher KJ, Petersdorf KG, et al., editors. Harrison's Principles of Internal Medicine. 11th ed. New York: McGraw-Hill, 1987: 1995-2000
6. Bryson HM, Sorkin EM. Cladribine: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in haematological malignancies. Drugs 1993 Nov; 46: 872-94
7. Bansil S, Cook SD, Rohowsky-Kochan C. Multiple sclerosis: immune mechanism and update on current therapies. Ann Neurol 1995 May; 37 Suppl. 1: S87-S101
8. Hafler DA, Weiner HL. MS: a CNS and systemic autoimmune disease. Immunol Today 1989; 10 (3): 104-7
9. Spasokukotskaja T, Arnér ESJ, Brosjö O, et al. Expression of deoxycytidine kinase and phosphorylation of 2-chlorodeoxyadenosine in human normal and tumour cells and tissues. Eur J Cancer A 1995; 31A (2): 202-8
10. Perzer AL, Bulgen R, Zilian U, et al. Inhibitory effect of 2-chlorodeoxyadenosine on granulocytic, erythroid, and T-lymphocytic colony growth. Blood 1991 Nov 15; 78: 2583-7
11. King KM, Cass CE. Membrane transport of 2-chloro-2'-deoxyadenosine and 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine is required for cytotoxicity [abstract]. 85th Annual Meeting of the American Association for Cancer Research 1994 Apr 10: 577
12. Kawasaki H, Carrera CJ, Piro LD, et al. Relationship of deoxycytidine kinase and cytoplasmic 5'-nucleotidase to the chemotherapeutic efficacy of 2-chlorodeoxyadenosine. Blood 1993 Feb 1; 81: 597-601
13. Staub M, Sasvári-Szekely M, Spasokukockaja T, et al. 2-Chlorodeoxyadenosine increases the activity of deoxycytidine kinase in human lymphocytes [abstract]. Pharm World Sci 1995 Sep 22: 17 Suppl. K: 12
14. Robertson LE, Chubb S, Meyn RE, et al. Induction of apoptotic cell death in chronic lymphocytic leukemia by 2-chloro-2'-deoxyadenosine and 9-β-D-arabinosyl-2-fluoroadenine. Blood 1993 Jan 1; 81: 143-50
15. Gartenhaus RB, Wang P, Hoffman M, et al. The induction of p53 and WAF1/CIP1 in chronic lymphocytic leukemia cells treated with 2-chlorodeoxyadenosine. J Mol Med 1996 May; 74: 143-7
16. Idink CAM, Schaafsma MR, Vermes I, et al. 2-Chlorodeoxyadenosine (2CDA) induced apoptosis in patients with hairy cell leukemia [abstract]. Proc Am Soc Clin Oncol 1995 Mar; 15: 89
17. Hentosh P, Grippo PJ. 2-Chloroadenine residues in DNA alter binding of DNA polymerase and human transcription factor *in vitro* [abstract]. 84th Annual Meeting of the American Association for Cancer Research 1993 Mar; 34: 352
18. Henderson DS, Otway RT, Crisp LB, et al. The mechanism of toxicity of 2-chlorodeoxyadenosine against human leukaemias [abstract]. Aust N Z J Med 1996 Apr; 26: 319
19. Beutler E, Sipe JC, Romine JS, et al. The treatment of chronic progressive multiple sclerosis with cladribine. Proc Natl Acad Sci U S A 1996 Feb 20; 93: 1716-20
20. Beutler E, Sipe J, Romine J, et al. The treatment of multiple sclerosis and other autoimmune diseases with cladribine. Semin Hematol 1996 Jan; 33: 45-52
21. Beutler E, Koziol JA, McMillan R, et al. Marrow suppression produced by repeated doses of cladribine. Acta Haematol 1994; 91: 10-5
22. Grieb P, Stelmasiak Z, Solski J, et al. Effect of repeated treatments with cladribine (2-chlorodeoxyadenosine) on blood counts in multiple sclerosis patients. Arch Immunol Ther Exp 1995; 43: 323-7
23. Stelmasiak Z, Solski J, Jakubowska B, et al. Safety of cladribine treatment in multiple sclerosis [abstract]. J Neuroimmunol 1995 Suppl. 1: 17
24. Liliemark J. The clinical pharmacokinetics of cladribine. Clin Pharmacokinet 1997 Feb; 32: 120-31
25. Albertioni F, Herngren L, Juliusson G, et al. Protein binding of 2-chloro 2'-deoxyadenosine (cladribine) in healthy subjects and in patients with leukaemia. Eur J Clin Pharmacol 1994; 46 (6): 563-4

26. Ortho Biotech Inc. LeustatinR (cladribine) injection. Prescribing information. Ortho Biotech, Raritan, NJ 08869, USA
27. Leustat injection. In: *ABPI compendium of data sheets and summaries of product characteristics. 1996-97* ed. London: Datapharm Publications Limited, 1996; 453-4
28. Liliemark J, Albertioni F, Hassan M, et al. On the bioavailability of oral and subcutaneous 2-chloro-2'-deoxyadenosine in humans: alternative routes of administration. J Clin Oncol 1992 Oct; 10: 1514-8
29. Albertioni F, Juliusson G, Liliemark J. On the pharmacokinetics of 2-chloro-2'-deoxyadenosine (CdA): metabolism to 2-chloroadenine (CAde) and renal excretion after intravenous and oral administration [abstract]. Proc Am Soc Clin Oncol 1994 Mar; 13: 157
30. Beutler E. Cladribine (2-chlorodeoxyadenosine) [see comments]. Lancet 1992 Oct 17; 340: 952-6
31. Ortho Biotech. Raritan, NJ, USA. Data on file
32. Grieb P, Ryba M, Stelmasiak Z, et al. Cladribine treatment of multiple sclerosis [letter]. Lancet 1994 Aug 20; 344: 538
33. Stelmasiak Z, Solski S, Nowicki J, et al. A pilot trial of cladribine (2-chlorodeoxyadenosine) in remitting-relapsing multiple sclerosis. Med Sci Monit 1998; 4 (1): 1-5
34. Romine JS, Sipe JC, Koziol JA, et al. Cladribine: use in therapy of multiple sclerosis. Biodrugs 1997 May; 7: 386-93
35. Sipe JC, Romine JS, Koziol JA, et al. Cladribine in treatment of chronic progressive multiple sclerosis. Lancet 1994 Jul 2; 344: 9-13
36. Rice G, Cladribine Study Group. Cladribine and chronic progressive multiple sclerosis: the results of a multicenter trial [abstract]. Neurology 1997 Jun; 48: 1730
37. Bekiesinska-Figatowska M, Walecki J, Stelmasiak Z. The value of magnetic resonance imaging in diagnosis and monitoring of treatment in multiple sclerosis. Acta Neurobiol Exp Warsz 1996; 56: 171-6
38. Beutler E, Sipe JC, Romine JS, et al. Cladribine therapy for chronic progressive and relapsing/remitting multiple sclerosis [abstract]. J Invest Med 1997 Mar; 45: 287A
39. Koziol JA, Frutos A, Sipe JC, et al. A comparison of two neurologic scoring instruments for multiple sclerosis. J Neurol 1996 Mar; 243: 209-13
40. Polman CH, Hartung H-P. The treatment of multiple sclerosis: current and future. Curr Op Neurol Neurosurg 1995 Jun; 8: 200-9
41. Miller DH, Albert PS, Barkhof F, et al. Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. Ann Neurol 1996; 39: 6-16
42. Lublin FD, Reingold SC, National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Guidelines for clinical trials of new therapeutic agents in multiple sclerosis. Relations between study investigators, advisors, and sponsors. Neurology 1997 Mar; 48: 572-4
43. Baltz JK, Montello MJ. Cladribine for the treatment of hematologic malignancies. Clin Pharm 1993 Nov; 12: 805-13
44. Sipe JC, Romine JS, Koziol JA, et al. Development of cladribine treatment in multiple sclerosis. Multiple Sclerosis 1996 Jul; 1 (6): 343-7
45. Saven A, Kawasaki H, Carrera CJ, et al. 2-Chlorodeoxyadenosine dose escalation in nonhematologic malignancies. J Clin Oncol 1993 Apr; 11: 671-8
46. Reid JM, Bagniewski PG, Richardson RL, et al. Clinical pharmacology of 2-chlorodeoxy-adenosine in patients with solid tumors, chronic leukemia or lymphoma [abstract]. Ann Oncol 1994; 5 Suppl. 5: 174
47. Polman CH, Koetsier JC. Cladribine treatment of multiple sclerosis [letter]. Lancet 1994 Aug 20; 344: 537
48. Sipe J, Koziol J, Romine J, et al. Cladribine treatment of multiple sclerosis. Reply [letter]. Lancet 1994 Aug 20; 344: 537-8
49. Ebers GC. Treatment of multiple sclerosis. Lancet 1994 Jan 29; 343: 275-9
50. Holliday SM, Benfield P. Interferon-β-1a: a review of its pharmacological properties and therapeutic potential in multiple sclerosis. Biodrugs 1997 Oct; 8: 317-30
51. Ebers G, Paty DW, Oger J, et al. The PRISMS study: prevention of relapses and disability by interferon-beta 1a, Rebif®, subcutaneously in multiple sclerosis [poster]. 13th Congress of the European Committee for Treatment and Research in Multiple Sclerosis. Istanbul, Turkey. 2-5 Nov, 1997
52. Faulds D, Benfield P. Interferon beta-1b in multiple sclerosis. An initial review of its rationale for use and therapeutic potential. Clin Immunother 1994 Jun; 1: 79-87
53. Lea AP, Goa KL. Copolymer-1: a review of its pharmacological properties and therapeutic potential in multiple sclerosis. Clin Immunother 1996 Oct; 6: 319-31

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Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy

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Multiple sclerosis is a chronic inflammatory disease of the nervous system in which a T-cell-mediated inflammatory process is associated with destruction of myelin sheaths. Although demyelination is the primary event, axons are also destroyed in the lesions, and the loss of axons correlates with permanent functional deficit. Here, we discuss evidence that demyelination and axonal destruction follow different pathogenetic pathways in subgroups of patients. This might, at least in part, explain the heterogeneity in genetic susceptibility, clinical presentation and response to treatment observed between individuals.

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Multiple sclerosis (MS) is the most common neurological disorder in young adults in the developed world. It is a chronic inflammatory disease of the central nervous system (CNS), which leads to large focal lesions of PRIMARY DEMYELINATION (see Glossary) with relative axonal preservation. It is considered to be an autoimmune disease that is induced when T HELPER 1 CELLS (Th1) recognize components of the myelin sheath. Activated, autoreactive T cells within the lesions are believed to drive the chronic inflammatory process and activate local or hematogenous macrophages that destroy myelin. However, we suggest that this pathogenetic scheme is oversimplified and cannot explain lesion formation. It is known that T-cell populations other than classical Th1 cells contribute to inflammation in MS and that amplification of demyelination in a chronic inflammatory reaction in the brain requires additional factors. Furthermore, the patterns of demyelination are different between different subgroups of MS patients, which suggests that the disease is heterogeneous. Depending upon the patient, factors that amplify demyelination can be either antibodies directed against surface components of myelin or factors that impair the metabolism of myelin-supporting oligodendrocytes. Although these insights are based mainly on pathological studies of biopsy or autopsy tissue, new clinical and paraclinical markers to distinguish these different patterns of demyelination look promising. The heterogeneity in the pathology of MS could form the basis of both the polygenic nature and the profound heterogeneity of the disease with respect to clinical presentation and response to therapy.

Basic features of MS lesions

The pathology of MS is distinguished from that of other inflammatory diseases of the nervous system by the presence of large, multifocal, demyelinated plaques with reactive glial scar formation[1,2]. This demyelinating process is accompanied by an inflammatory reaction with infiltrates composed mainly of T cells and macrophages. Although myelin sheaths are the primary target of tissue destruction, axons, nerve cells and astrocytes are also affected, although to a lesser degree. Active lesions, defined by the ongoing destruction of myelin, are heavily infiltrated by macrophages and activated microglial cells. These cells are closely associated with the disintegrating myelin sheaths and are responsible for the uptake and removal of myelin debris.

Although MS is a primary demyelinating disease with relative sparing of axons, the emphasis is on the term 'relative'. Acute axonal injury is frequent in actively demyelinating MS lesions[3], and this leads to a 50–70% reduction in neurite density in chronic plaques, compared with normal tissue. Although demyelination can be repaired, at least in part, by remyelination[2], axonal destruction is irreversible. Thus, in MS patients, the relapsing–remitting functional impairment is caused mainly by inflammation and demyelination, whereas the accumulation of an irreversible neurological deficit is caused mainly by axonal destruction and loss.

Is Th1-mediated autoimmunity against myelin the cause of inflammation in MS?

The pathology of inflammation in MS lesions is consistent with a T-cell-mediated immune reaction, leading to the recruitment of hematogenous macrophages and activation of microglia[4]. This is similar to the pathology of experimental autoimmune encephalomyelitis (EAE), a disease induced by immunization of animals with CNS tissue, myelin or myelin proteins. Several features of MS lesions suggest that, as in EAE, the inflammatory process in MS is driven by a Th1-mediated autoimmune response. For example, the number of autoreactive T cells with the cytokine spectrum of Th1 cells is increased in blood of MS patients compared with controls. Furthermore, within actively demyelinating lesions, Th1-related cytokines, such as interferon γ, tumour necrosis factor α (TNF-α) or interleukin 2, are expressed in invading leukocytes and local glial cells[5]. The spectrum of chemokines and chemokine receptors is also consistent with a Th1-driven inflammatory response[6], and MS is

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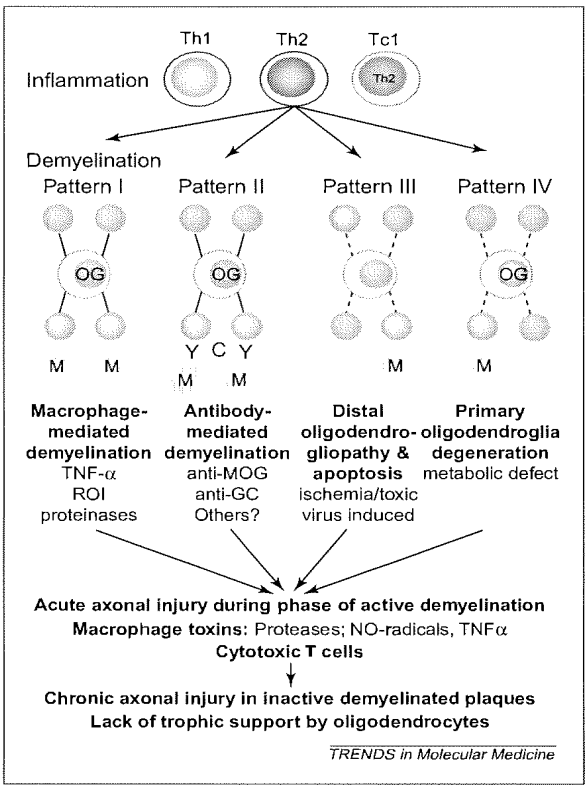

Fig. 1. Summary of pathogenetic mechanisms involved in the formation of multiple sclerosis lesions. Inflammation: evidence indicates that T helper 1 (Th1) cells have a role in inducing inflammatory reactions in the central nervous system. Proinflammatory cytokines released from Th1 cells activate macrophages, which are responsible for the majority of demyelination and axonal injury. In addition, however, T helper 2 (Th2) cells and cytotoxic, CLASS I-RESTRICTED CYTOTOXIC T CELLS (TC1) might modify the outcome of the lesions. Demyelination: myelin sheaths and oligodendrocytes (OG) can be destroyed, possibly by different mechanisms in different individuals. This results in distinctly different patterns of demyelination in active lesions. Demyelination may be induced by macrophages (M) and/or their toxic products (resulting in pattern I), by specific demyelinating antibodies and complement (C, resulting in pattern II), by degenerative changes in distal processes, in particular those of periaxonal oligodendrocytes (distal oligodendrogliopathy), followed by apoptosis (resulting in pattern III) or by a primary degeneration of oligodendrocytes followed by myelin destruction (resulting in pattern IV). Possible mediators of myelin and oligodendrocyte destruction include tumor necrosis factor α (TNF-α), reactive oxygen intermediates (ROI), antibodies against myelin oligodendrocyte glycoprotein (anti-MOG) or galactocerebroside (anti-GC). Axonal injury: axonal injury follows acute destruction of myelin sheaths. In the active phase of demyelination, axonal injury is likely to be induced by macrophage toxins or by the direct effects of cytotoxic T cells. The chronic axonal injury observed inactive plaques may be caused by a lack of trophic support by glial cells, such as oligodendrocytes, but could also involve inflammatory mediators, produced by macrophages, that persist in most active chronic lesions.

associated with certain major histocompatibility complex class II haplotypes[7]. Thus, it is generally believed that the immunological mechanisms responsible for inflammation in MS are similar to those in EAE, an experimental paradigm of Th1-mediated autoimmune disease, and that immunomodulatory therapeutic strategies that work in EAE should also be beneficial in MS. Unfortunately, in many instances this approach has been disappointing[8].

Glossary

Primary demyelination: destruction of myelin sheaths with relative sparing of axons.
T helper 1 cells (Th1): MHC class II-restricted T cells that secrete a spectrum of cytokines including interleukin 2, interferon γ and lymphotoxin α, and elicit a delayed-type hypersensitivity reaction.
T helper 2 cells (Th2): MHC class II-restricted T cells that secrete mainly interleukins 4, 5, and 6. They stimulate antibody production and are involved in allergic reactions.
Class I-restricted cytotoxic T cells (Tc1): cells that secrete a similar spectrum of cytokines to Th1 cells.

One possible reason for this might be that the pathogenesis of MS lesions is more complex than a pure Th1-mediated CNS autoimmune disease. There is evidence that cells other than classical Th1 cells contribute to the inflammatory response in MS lesions (Fig. 1). Numerically, CD8, class I-restricted T cells outnumber CD4 cells[9]. Furthermore, class I-restricted T cells are predominant at the site of tissue destruction in actively demyelinating lesions, whereas CD4 cells are retained mainly in perivascular inflammatory infiltrates[10]. Recent studies using PCR to analyze single cells show that clonal expansion is much more prominent in the CD8 population than in the CD4 T-cell population[10]. Additionally, the extent of axonal injury and tissue destruction correlates better with the number of macrophages and CD8 cells in the lesions than with CD4 cells. Taken together, these data suggest that class I-restricted T cells could play an important role in the pathogenesis of MS.

Another feature of inflammation in MS that is incompatible with the concept of a purely Th-1-mediated disease is the abundance of granulocytes and eosinophils in active lesions of acute and fulminate variants, in particular in Marburg's type of acute MS and Devic's type of neuromyelitis optica. An inflammatory reaction similar to that found in Devic's disease can be induced by transfer of Th2-polarized autoreactive T cells to immunocompromised animals[11]. These data suggest that T HELPER 2 CELL (TH2)-mediated mechanisms might contribute to inflammation in subsets of patients with MS (Ref. 12).

Additional demyelinating amplification factors are required to form demyelinated plaques

In most vertebrates, including rats, guinea pigs and primates, pure T-cell-mediated inflammation of the brain does not lead to demyelination. An exception to this is the mouse, in which extensive activation of macrophages and microglia in EAE, or in certain transgenic models, is associated with primary destruction of myelin. In these mouse models, signaling through TNF receptor 1 is required for the destruction of myelin and oligodendrocytes[13].

Pure inflammatory T-cell-mediated encephalomyelitis can, however, become an MS-like demyelinating disease in the presence of specific demyelinating amplification factors. One such factor, which has been extensively characterized recently, is

Box 1. Multiple sclerosis cases included in a study of the heterogeneity of multiple sclerosis lesions[a]

As the mechanisms of demyelination can only be studied in MS lesions in which myelin is at the stage of disintegration, observations on the heterogeneity of demyelination are from studies of active lesions (Table I). Because active lesions are more common in patients with fulminate acute disease, such as that in acute MS or in biopsies from early MS, these conditions are over-represented in our material. Thus, the study contains an inherent bias towards patients with exceptionally severe disease. Also included, however, are samples from 18 patients with different forms of chronic MS, which have a similar spectrum of lesional patterns.

Undoubtedly, the full spectrum of pathology within active MS lesions is best recognized following autopsy, where large areas of the lesions plus surrounding brain tissue can be analyzed. However, the different patterns of demyelination, described here (Table II), can be identified unequivocally even in small stereotactic biopsies, provided an area of active demyelination is present and appropriate care is taken in staging of the lesions.

References

a Lucchinetti, C. *et al.* (2000) Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination. *Ann. Neurol.* 47, 707–717

b Griot, C. *et al.* (1990). Selective degeneration of oligodendrocytes mediated by reactive oxygen species. *Free Radic. Res. Commun.* 11, 181–193

c Brück, W. *et al.* (1995) Monocyte/macrophage differentiation in early multiple sclerosis. *Ann. Neurol.* 38, 788–796

Table I. Multiple sclerosis cases included in this study[b]

Definition of clinical MS courses	Number of cases
Acute multiple sclerosis (Marburg's Type): Fulminate inflammatory demyelinating disease with typical multiple sclerosis pathology leading to patient's death within one year after onset.	14 autopsies
Early multiple sclerosis (biopsies): Patients with severe acute neurological disease with atypical clinical and MRI presentation (e.g. large monofocal lesions; no intrathecal immunoglobulin synthesis); Biopsy taken generally during the first months after disease onset with pathology of inflammatory demyelinating disease resembling MS; 77% of these patients develop clinically definite MS in follow up (average follow up time: 37 months).	51 biopsies
Chronic active multiple sclerosis: Patients with clinically definite chronic multiple sclerosis (relapsing-remitting; primary progressive or secondary progressive); At least one actively demyelinating lesion present within the CNS.	18 autopsies
Chronic inactive multiple sclerosis: Patients with clinically definite multiple sclerosis; (relapsing-remitting; primary progressive or secondary progressive); No actively demyelinating lesions in whole CNS.	not included

Table II. Defining demyelinating activity[c]

Definition of demyelinating activity	Number of lesions
Active lesions: Lesions with infiltration by macrophages, which contain intracytoplasmic granules, immunoreactive for all myelin proteins, including minor myelin components such as myelin oligodendrocyte glycoprotein	173 lesions in autopsies 62 lesions in biopsies
Inactive lesions: Demyelinated or remyelinated lesions, with or without macrophage infiltration; Macrophages do not contain degradation products, immunoreactive for minor myelin proteins;	152 lesions in autopsies 9 lesions in biopsies

the presence of demyelinating antibodies directed against epitopes expressed on the surface of myelin sheaths and oligodendrocytes[14]. In this experimental paradigm, brain inflammation, mediated by T cells, induces local activation of hematogenous macrophages or microglia, and impairs the blood–brain barrier. The latter allows circulating demyelinating antibodies and complement components to enter the CNS and destroy myelin, either by complement activation or an antibody-dependent cellular cytotoxicity reaction. The pathological hallmark of such lesions is the local precipitation of the lytic terminal complement complex on the surface of myelin sheaths and oligodendrocytes.

However, other mechanisms that have been identified could induce myelin damage during the course of an inflammatory process in the CNS. These include direct T-cell-mediated cytotoxicity[15] and metabolic impairment of oligodendrocytes through ischemia[16], toxins[17] or virus infection[18]. Thus, a major challenge for future research, is to define which of these mechanisms operate in actively demyelinating MS plaques.

Heterogenous patterns of demyelination in MS

A major restriction of pathogenetic studies of MS is the limited material available from actively demyelinating lesions, and it has required a large international effort to collect sufficient specimens to perform such a study[19,20] (Box 1). A detailed immunopathological investigation of this material has revealed a profound heterogeneity in the patterns of demyelination between different patients, although active plaques from the same patient were very

Table 1. Essential characteristics of different patterns of demyelination in multiple sclerosis

Pattems of demyelination	Pathology	Putative mechanisms
(I) Macrophage mediated	Perivenous distribution of lesions; Radial expansion of the lesions; Inflammatory infiltrates composed of T-cells and macrophages; Activated macrophages and microglia associated with degenerating myelin.	T-cell-mediated inflammation with macrophage/microglia activation; Demyelination induced by macrophage toxins.
(II) Antibody mediated	Similar lesions as in I but additional deposition of immunoglobulin and activated complement at sites of active myelin destruction	T-cell-mediated inflammation with macrophage/microglia activation; Complement mediated lysis of antibody-targeted myelin
(III) Distal oligodendrogliopathy	Inflammation by T-cells and macrophages; Small vessel vasculitis with endothelial cell damage and microvessel thrombosis; Degeneration of distal oligodendrocyte processes, followed by oligodendrocyte apoptosis and demyelination	T-cell-mediated small vessel vasculitis with secondary ischemic damage of the white matter
(IV) Primary oligodendrocyte damage with secondary demyelination	Similar lesion as in (I), but prominent oligodendrocyte degeneration in a small rim of periplaque white matter	T-cell-mediated inflammation with macrophage/microglia activation; Demyelination induced by macrophage toxins on the background of metabolically impaired oligodendrocytes; Genetic defect of oligodendrocytes?

similar. All actively demyelinating lesions were associated with an inflammatory process, with the inflammatory infiltrates composed mainly of T cells and macrophages. Despite the similarities in the inflammatory reaction the lesions segregated into four patterns of myelin destruction (Table 1, Figs 1,2).

Pattern I (macrophage-associated demyelination) closely resembles myelin destruction in mouse models of autoimmune encephalomyelitis. In these models, toxic products of activated macrophages, such as TNF-α (Ref. 13) or reactive oxygen species, are mainly responsible for the destruction of myelin sheaths[21]. Lesions similar to pattern II (antibody-mediated demyelination) are found in models of EAE that are induced by sensitization with myelin oligodendrocyte glycoprotein (MOG). In this model, demyelination is induced by cooperation between encephalitogenic T cells, which are responsible for inflammation, and demyelinating anti-MOG antibodies[14]. So far, patterns III and IV have not been identified in experimental models of demyelinating disease. Distal oligodendrogliopathy-associated demyelination (pattern III), however, is commonly found in virus-induced human white-matter diseases[22] and is also seen in the penumbra region of white-matter strokes (Rauschka *et al.*, unpublished). Preliminary evidence from our laboratory suggests that white-matter ischemia is a major pathogenetic factor for demyelination and tissue damage in such lesions. The mechanisms responsible for pattern IV lesions (primary oligodendrocyte degeneration), which are the most infrequent in the MS population and are restricted to a subset of patients with primary progressive disease[20], are not clear. It is tempting to speculate that, in these patients, a genuine metabolic disturbance of oligodendrocytes could render these cells particularly vulnerable to the toxic action of inflammatory mediators.

The heterogenous patterns of pathology so far could only be related to a specific clinical disease variant in patients with most acute and fulminate disease course. All patients with Devic's type of neuromyelitis optica had antibody-mediated tissue damage[23] (pattern II), whereas all patients with Balo's type of concentric lesions had lesions of distal oligodendrogliopathy (pattern III). Furthermore, primary oligodendrocyte degeneration (pattern IV) has only been found in a small subset of patients with primary progressive disease. Except for this segregation in the most severe disease variants, no specific association between the pattern of demyelination and clinical disease was found.

Axonal injury and loss: a consequence of inflammatory demyelination

Early descriptions of the pathology of MS, published in the early 1900s, emphasized the functional importance of axonal destruction in the lesions, which led to secondary (Wallerian) tract degeneration and brain atrophy[3]. This aspect of MS pathology has received increased attention recently, as serial magnetic resonance imaging (MRI) investigations indicate that axonal loss within the lesions and brain atrophy correlate with permanent, progressive functional deficits. Axonal injury in MS plaques occurs in two stages[24]. A high incidence of acute axonal injury is found in lesions during the active stage of myelin destruction[25,26]. Thus, even during the earliest stages of the disease, every newly formed plaque is associated

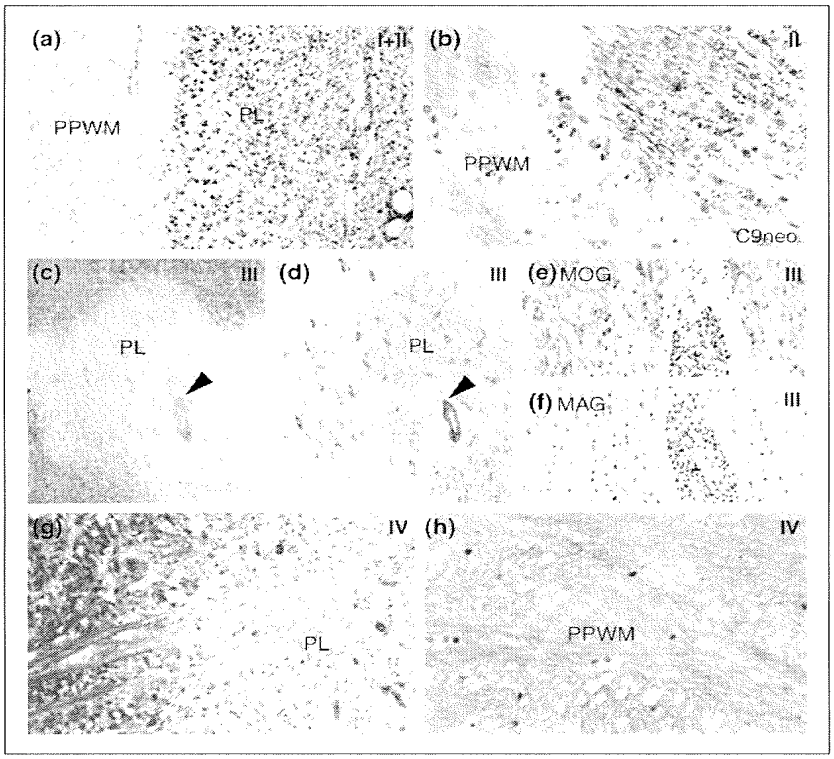

Fig. 2. Histopathology of different patterns of demyelination in multiple sclerosis. (a) Actively demyelinating lesion following patterns I and II. The active plaque (PL) is filled with activated macrophages and microglia. There is a sharp demarcation between the actively demyelinating lesions and the periplaque white matter (PPWM). Immunocytochemistry for CD68 (to identify activated macrophages/microglia). Magnification × 200. (b) Actively demyelinating plaque of pattern II that shows massive deposition of complement C9neo-antigen (brown staining) on degenerating myelin sheaths and in myelin degradation products taken up by macrophages in the zone of active demyelination (ADM). There is faint C9neo reactivity on myelin sheaths in the PPWM. Immunocytochemistry for C9neo-antigen. Magnification × 500. (c) Actively demyelinating lesion following pattern III. Myelin staining using Luxol fast blue shows an ill-demarcated demyelinated plaque (PL). In the centre of the lesion is an inflamed blood vessel surrounded by a small rim of preserved myelin (arrow). Magnification × 30. (d) The same lesion as shown in (c) stained with the leukocyte marker CD45. Myelin around the central vessel has a lower density of inflammatory cells compared to the rest of the lesion (arrow). In addition, this lesion has an indistinct boundary compared with the lesion in panel (a). Immunocytochemistry for CD45. Magnification × 30. (e) Higher magnification of the area indicated by the arrow in panels (c) and (d) stained for myelin-oligodendrocyte glycoprotein (MOG, brown staining). There are numerous MOG-reactive fibers preserved in the lesion. Magnification × 300. (f) Higher magnification of the area indicated by the arrow in panels (c) and (d) stained for myelin associated glycoprotein (MAG). There is very little MAG immunoreactivity. Magnification × 300. (g) Actively demyelinating lesion following pattern IV. The plaque contains numerous macrophages containing myelin degradation products (stained blue with the Luxol fast blue myelin stain) and has a sharply demarcated edge. Magnification × 300. (h) The periplaque white matter of the lesion in (g). The myelin appears vacuolated and contains numerous oligodendrocytes with fragmented DNA (black nuclei) identified using an *in situ* tailing reaction for DNA fragmentation. Magnification × 400.

with a significant loss of axons. In this phase, the extent of axonal injury correlates with the number of macrophages and class I-restricted T cells in the lesions, and is apparently mediated by toxic products of macrophages and T cells. A few mediators have been identified, such as nitric oxide and proteases, that can induce axonal injury *in vitro* or *in vivo*[27,28]. In addition to axonal degeneration in active plaques, there is also a low level of continuous axonal destruction and loss in chronic inactive demyelinated plaques[24]. This is not seen in inactive, remyelinated lesions, so the lack of trophic support by oligodendrocytes in demyelinated lesions might render axons vulnerable to progressive damage. Although the initial event that triggers axonal damage can differ, the final pathway of axonal destruction appears to be similar in all conditions of cerebral damage; alterations in ion-channel permeability disturb calcium homeostasis within the axons, which leads to activation of calcium-dependent proteases, local degradation of cytoskeletal elements, blockade of axonal transport and, finally, axonal disruption[29].

The extent of axonal damage in MS lesions is variable and depends upon the severity of the inflammatory process during the active stage of demyelination. Other factors that influence the degree of axonal injury are the pathogenetic mechanism of demyelination and, possibly, heterogeneity in the susceptibility of individual patients. Within the MS population, axonal loss appears to be most important in patients with either primary or secondary progressive disease courses.

These data provide support for the concept that the mechanism of demyelination and tissue destruction is heterogenous between different subgroups of patients, and could have profound consequences for our understanding of disease pathogenesis and for the future design of novel therapeutic strategies.

Is inflammation in MS lesions always deleterious?

As previously discussed, T-cell-mediated inflammation is the apparent driving force behind the pathological process in MS lesions. More recent data, however, indicate that inflammation might also be neuroprotective, or have a role in the repair of damaged tissue within the CNS. For example, autoimmune T cells protect neurons in the optic nerve from secondary degeneration after injury by partial crushing[30]. The presence of macrophages stimulates remyelination in organ culture[31], an observation that could be particularly relevant for MS lesions. Furthermore, inflammatory cells within MS lesions synthesize neurotrophic factors, such as brain-derived neurotrophic factor[32]. These data suggest that the inflammatory response within demyelinated plaques of MS patients might also have a role in the repair process and, thus, complete blockage of all inflammatory processes within the lesions could be counterproductive.

Clinical identification of subgroups of MS patients

The heterogenous patterns of demyelination in individuals with MS patients are defined by the pathology of active lesions. However, we expect that clinical diagnosis and therapy would be aided by developing clinical and paraclinical markers to identify patient subgroups according to their pathogenetic pathways of lesion formation. As the association between clinical forms of the disease and pathological subtypes is limited, this approach will largely depend upon the development of suitable paraclinical markers.

It is possible that subsets of MS patients will be identified using MRI and magnetic resonance

Outstanding questions

- What immunological and neurobiological mechanisms underlie the heterogenous patterns of MS pathology?
- Which are the most suitable clinical and paraclinical markers to define patient subgroups with different pathogenetic pathways in the formation of demyelinated plaques?
- Do patients with different pathogenetic pathways of demyelination require subtype-specific therapy?
- Are recent therapies developed against specific mechanisms of demyelination or axonal injury effective in MS patients?

spectroscopy. These techniques focus on two major issues: determining the imaging correlate of basic histopathological MS features, such as inflammation, demyelination–remyelination, gliosis and axonal loss; and identifying different demyelination pathways. From their pathological appearance[20], it is predicted that pattern I and II lesions, which reflect classical autoimmunity, will be characterized by their sharp borders, and early and pronounced contrast enhancement as a reflection of damage to the blood–brain barrier. By contrast, lesions that involve oligodendrocytes (patterns III and IV) expand more diffusely into the white matter. Thus, these lesions have ill-defined borders and less pronounced and delayed blood–brain barrier damage. Lesions with these features have been described in subgroups of MS patients[33] and preliminary evidence indicates that they correlate with the respective pathological patterns.

Cases of MS in which antibody-mediated demyelination occurs could, additionally, be identified by the presence of serum-demyelinating antibodies. Such antibodies have been found in the serum of a subset of MS patients[34]. One of the major targets of demyelinating antibodies is MOG. Although anti-MOG antibodies occur in serum and cerebrospinal fluid of MS patients, a classification of MS patient subsets on the basis of their presence or absence is not yet possible[35,36]. An additional complication is that these antibodies are also found in control patients without MS and in patients with other neurological diseases. Furthermore, not all anti-MOG antibodies induce demyelination *in vivo* and *in vitro*: most anti-MOG antibodies recognize regions of MOG that are not exposed on the extracellular surface of oligodendrocytes and are therefore not targets for antibodies *in vivo*. Lastly, MOG is only one of the possible target antigens for antibody-mediated demyelination. More precise analysis of demyelinating anti-MOG antibodies, and the identification of other potential targets of pathogenic autoantibodies, should lead to tools that allow a more detailed categorization of MS patient subsets.

Consequences of MS therapy

Demyelination in MS develops by a T-cell-driven inflammatory process. Thus, the primary nature of inflammation is undisputed and will remain central for treatments that modulate the immune system[8]. There are, however, several aspects that limit the therapeutic efficacy of strategies directed exclusively against the inflammatory component of the disease. Currently, immune suppression is unable to stop the inflammatory reaction in the CNS and immune modulatory regimes using interferon β or co-polymer I decrease, but do not abolish, inflammation. It is not possible to intervene more specifically in the inflammatory process because neither the trigger of inflammation (virus induced versus autoimmunity) nor the specific target antigen in the CNS of affected patients is known. It should also be remembered that, in MS, evidence that inflammation is driven by a Th1 response is circumstantial, and a role for other T cells, for example a response mediated by class-I-restricted cells, is likely.

These uncertainties indicate the importance of identifying supplementary therapeutic strategies to prevent demyelination and tissue destruction in MS. Possible approaches include blocking macrophage responses or specific macrophage toxins, the elimination of specific demyelinating antibodies, neuroprotective therapies to prevent axonal injury or the consequences of brain ischemia, and stimulation of remyelination. Although attractive conceptually, these strategies have so far failed. It is possible that this is because of the heterogeneity in the pathogenetic mechanisms that leads to the formation of demyelinated plaques in this disease, which we have described earlier.

Thus, a major challenge for MS research is to develop paraclinical markers that identify the heterogenous pathogenetic components involved in the formation of MS plaques in individuals at different stages of their disease. This could lead to the stratification of MS patients into smaller subgroups with common, defined mechanisms of inflammation, demyelination and tissue damage, and to subtype-specific therapy.

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References

1 Lassmann, H. (1998) Multiple sclerosis pathology. In *McAlpine's Multiple Sclerosis* (3rd edn) (Compston, A., ed.), Churchill Livingstone
2 Prineas, J.W. (1985) The neuropathology of multiple sclerosis. In *Handbook of Clinical Neurology* (Vol 47. Demyelinating diseases) (Koetsier, J.C., ed.), pp. 337–395, Elsevier Science
3 Kornek, B. and Lassmann, H. (1999) Axonal pathology in multiple sclerosis: a historical note. *Brain Pathol.* 9, 651–656
4 Wekerle, H. (1998) Immunology of multiple sclerosis. In *McAlpine's Multiple Sclerosis* (3rd edn) (Compston, A., ed.), Churchill Livingstone
5 Merrill, J.E. (1992) Proinflammatory and antiinflammatory cytokines in multiple sclerosis and central nervous system acquired immunodeficiency syndrome. *J. Immunother.* 12, 167–170
6 Sorensen, T.L. *et al.* (1999) Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J. Clin. Invest.* 103, 807–815
7 Kalman, B. and Lublin, F.D. (1999) The genetics of multiple sclerosis. A review. *Biomed. Pharmacother.* 53, 358–370
8 Hohlfeld, R. (1997) Biotechnological agents for the immunotherapy of multiple sclerosis: principles, problems and perspectives. *Brain* 120, 865–916
9 Booss, J. *et al.* (1983) Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. *J. Neurol. Sci.* 62, 219–232

10 Babbe, H. *et al.* (2000) Clonal expansion of CD8 T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J. Exp. Med.* 192, 393–404
11 Lafaille, J.J. *et al.* (1997) Myelin basic protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. *J. Exp. Med.* 186, 307–312
12 Rook, G.A.W. *et al.* (2000) Bacterial vaccines for the treatment of multiple sclerosis and other autoimmune diseases. *Immunol. Today* 21, 503–508
13 Probert, L. *et al.* (2000) TNFR1 signalling is critical for the development of demyelination and the limitation of T-cell responses during immune-mediated CNS disease. *Brain* 123, 2005–2019
14 Linington, C. *et al.* (1988) Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am. J. Pathol.* 130, 443–454
15 D'Souza, S.D. *et al.* (1996) Multiple sclerosis: Fas signaling in oligodendrocyte death. *J. Exp. Med.* 184, 2361–2370
16 Husain, J. and Juurlink, B.H. (1995) Oligodendroglial precursor cell susceptibility to hypoxia is related to poor ability to cope with reactive oxygen species. *Brain Res.* 698, 86–94
17 Ludwin, S.K. and Johnson, E.S. (1981) Evidence of a 'dying-back' gliopathy in demyelinating disease. *Ann. Neurol.* 9, 301–305
18 Rodriguez, M. (1995) Virus-induced demyelination in mice: 'dying-back' of oligodendrocytes. *Mayo Clin. Proc.* 60, 433–438
19 Lucchinetti, C. *et al.* (1999) A quantitative analysis of oligodendrocytes in multiple sclerosis lesions. A study of 117 cases. *Brain* 122, 2279–2295
20 Lucchinetti, C. *et al.* (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann. Neurol.* 47, 707–717
21 Griot, C. *et al.* (1990). Selective degeneration of oligodendrocytes mediated by reactive oxygen species. *Free Radic. Res. Commun.* 11, 181–193
22 Itoyama, Y. *et al.* (1982) Distribution of papovavirus, myelin-associated glycoprotein, and myelin basic protein in progressive multifocal leukoencephalopathy lesions. *Ann. Neurol.* 11, 396–407
23 Lucchinetti, C. *et al.* (2000) Humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Neurology* 54 (Suppl. 3), A259
24 Kornek, B. *et al.* (2000) Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive and remyelinated lesions. *Am. J. Pathol.* 157, 267–276
25 Trapp, B.D. *et al.* (1998) Axonal transsection in the lesions of multiple sclerosis. *New Engl. J. Med.* 338, 278–285
26 Ferguson, B. *et al.* (1997) Axonal damage in acute multiple sclerosis. *Brain* 120, 393–399
27 Kapoor, R. *et al.* (1999) Temporary axonal conduction block and axonal loss in inflammatory neurological disease. A potential role for nitric oxide? *Ann. New York Acad. Sci.* 893, 304–308
28 Anthony, D.C. *et al.* (1998) Matrix metalloproteinase expression in an experimentally-induced DTH model of multiple sclerosis in the rat CNS. *J. Neuroimmunol.* 87, 62–72
29 LoPachin, R.M. and Lehning, E.J. (1997) Mechanism of calcium entry during axon injury and degeneration. *Toxicol. Appl. Pharmacol.* 143, 233–244
30 Moalem, G. *et al.* (1999) Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. *Nat. Med.* 5, 49–55
31 Diemel, L.T. *et al.* (1998) Macrophages in CNS remyelination: friends or foe? *Neurochem. Res.* 23, 341–347
32 Kerschensteiner, M. *et al.* (1999) Activated human T cells, B cells and monocytes produce brain-derived neurotrophic factor (BDNF) *in vitro* and in brain lesions: A neuroprotective role of inflammation? *J. Exp. Med.* 189, 856–870
33 Filippi, M. and Rovaris, M. (2000) Magnetisation transfer imaging in multiple sclerosis. *J. Neurovirol.* 6 (Suppl. 2), S115–S120
34 Grundke-Iqbal, I. and Bornstein, M.B. (1980) Multiple sclerosis: serum gamma globulin and demyelination in culture. *Neurology* 30, 749–754
35 Lindert, R.B. *et al.* (1999) Multiple sclerosis: B- and T-cell responses to the extracellular domain of the myelin oligodendrocyte glycoprotein. *Brain* 122, 2089–2099
36 Reindl, M. *et al.* (1999) Antibodies against the myelin oligodendrocyte glycoprotein and the myelin basic protein in multiple sclerosis and other neurological diseases: a comparative study. *Brain* 122, 2047–2056
37 Brück, W. *et al.* (1995) Monocyte/macrophage differentiation in early multiple sclerosis. *Ann. Neurol.* 38, 788–796

Molecular basis of partial lipodystrophy and prospects for therapy

Robert A. Hegele

Lipodystrophy is characterized by altered partition of adipose tissue. Despite heterogeneous causes, which include genetic, autoimmune and drug-induced forms, lipodystrophy syndromes have similar metabolic attributes, including insulin resistance, hyperlipidemia and diabetes. The mechanisms underlying the insulin resistance are unknown. One form of lipodystrophy, namely Dunnigan-type familial partial lipodystrophy (FPLD) was shown to result from mutations in the *LMNA* gene, which encodes nuclear lamins A and C. Although the relationship between the mutations in the nuclear envelope and insulin resistance is unclear at present, these findings might eventually be shown to have relevance for the common insulin resistance syndrome and for drug-associated lipodystrophies.

The compensatory hyperinsulinemia that is required to maintain glucose tolerance in people with insulin resistance is associated with a cluster of metabolic abnormalities, which usually presents before the onset of diabetes mellitus[1,2]. This metabolic cluster is referred to as 'metabolic syndrome X' or 'insulin resistance syndrome'[1,2]. This disorder is frequently seen in individuals with android obesity, and is characterized by glucose intolerance, dyslipidemia and hypertension[1,2], which together contribute to accelerated atherosclerosis. Defining the underlying mechanisms of insulin resistance might help to develop preventative and/or treatment strategies. One approach to understand a common complex phenotype is to study a genetically extreme form. An extreme monogenic form of insulin resistance is DUNNIGAN-TYPE FAMILIAL PARTIAL LIPODYSTROPHY (FPLD) (see Glossary).

Clinical attributes of FPLD

In the 1940s, Lawrence reported a diabetic patient with atrophic fat stores and hyperlipidemia[3].

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Defining the clinical course of multiple sclerosis:

Results of an international survey

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Article abstract—Standardization of terminology used to describe the pattern and course of MS is essential for mutual understanding between clinicians and investigators. It is particularly important in design of, and recruitment for, clinical trials statistically powered for expected outcomes for given patient populations with narrowly defined entry criteria. For agents that prove safe and effective for MS, knowledge of the patient populations in definitive clinical trials assists clinicians in determining who may ultimately benefit from use of the medication. An international survey of clinicians involved with MS revealed areas of consensus about some terms classically used to describe types of the disease and other areas for which there was lack of consensus. In this report, we provide a summary of the survey results and propose standardized definitions for the most common clinical courses of patients with MS.

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The clinical course of MS may follow a variable pattern over time but usually can be characterized by either episodic acute periods of worsening (relapses, exacerbations, bouts, attacks), gradual progressive deterioration of neurologic function, or combinations of both. Although the terms used to describe these clinical forms have been used for many years,[1-7] there is no clear common meaning among clinicians for the terms used to describe forms or clinical stages of the disease. There is often lack of clarity about exactly which patient group is described. This creates real and potential problems in communication among investigators and in the design of, and recruitment for, multicenter clinical trials for new therapeutic agents that are based on expected clinical outcomes for defined patient groups and require narrow entrance criteria. The success of such trials may depend on the homogeneity of the population of MS patients entered into the study.

MS recently joined the growing ranks of treatable neurologic diseases, with reports of data on new therapies demonstrating clinical efficacy in pivotal clinical trials, such as interferon beta-1b (Betaseron, manufactured by Berlex Laboratories, Richmond, CA),[8] interferon beta-1a (Avonex, manufactured by Biogen, Cambridge, MA),[9] and copolymer I (Copaxone, manufactured by Teva Pharmaceutical Industries, Petah Tiqwa, Israel).[10] Many new trials begin each year. Although each of these studies uses a narrowly defined population of patients, clinicians look for guidance as to exactly which broader patient groups will likely benefit from treatment. The terms used to describe patient populations are crucial for this guidance.[11]

Informal discussions among clinicians and clinical researchers and consensus developed among investigators attending a 1994 MS clinical trials design workshop[12] revealed that there was no unanimous agreement on definitions for the various clinical subtypes of MS. This lack of unanimity resulted from the lack of clear biological markers to distinguish the various forms of MS. This required use of descriptive terms for these clinical subtypes, for which there was no consensus. These facts and perceptions underscored the need for a reassessment of the terminology used to describe MS and for more uniform definitions of MS clinical subtypes.

Methods. In the absence of agreed on biological markers, the Advisory Committee on Clinical Trials of New Agents in MS of the National Multiple Sclerosis Society (NMSS) (USA) undertook a survey to develop a perspective and consensus on definitions and terminology used to describe clinical outcomes and course patterns in patients with MS, to standardize terminology, and to facilitate a broader understanding of patient recruitment parameters in MS therapeutic trials. Those surveyed included 215 members of the international MS clinical research community, including members of the NMSS Medical Advisory

* See page 910 for Committee members.

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Board (current and past), members of the NMSS Advisory Committee on Clinical Trials of New Agents in MS, attendees at the 1994 International Workshop on Outcomes Assessment in MS Clinical Trials held in Charleston, South Carolina,[12] and other individuals known to be principally involved in MS clinical research and care. Survey forms developed by the authors in consultation with others with known interest in this issue were mailed in early January 1995, with respondents asked to reply by early February 1995. Of the 215 surveys, 125 (58%) were completed and returned.

The survey form asked respondents to choose among several possible clinical patterns commonly used to define the following MS disease courses and types: relapsing-remitting (RR), relapsing-progressive (RP), primary progressive (PP), secondary progressive (SP), benign, and malignant. The survey allowed respondents to provide their own definitions if they were not satisfied with those provided in the survey. Because respondents were asked to indicate all definitions that, in their view, applied to the disease type, more than 125 total responses were collected for some questions.

The results of the survey were collated and distributed to members of the NMSS Advisory Committee on Clinical Trials. After meeting, revising, and approving revised clinical definitions based on the survey responses, the definitions were presented to the executive committee of the NMSS Medical Advisory Board and the full NMSS Medical Advisory Board where additional clarifications and revisions were made. The final definitions, presented here, did not differ substantively from those in the initial survey document.

Results. *Clinical course definitions.*

Relapsing-remitting (RR) MS. The consensus definition is as follows: clearly defined disease relapses with full recovery or with sequelae and residual deficit upon recovery; periods between disease relapses characterized by a lack of disease progression (figure 1, a and b).

The defining elements of RR-MS are episodes of acute worsening of neurologic function followed by a variable degree of recovery, with a stable course between attacks. Although a clear majority (105/134) of responses included this definition, some (16/134) favored using the term relapsing-remitting only for those patients who fully recover between relapses. However, the lack of evidence for a biological difference between those who recover fully (figure 1a) and those who recover partially (figure 1b) and potential differences in the vigor with which one might seek to determine the extent of recovery (clinical examination, evoked potentials, and so on) favored the more inclusive definition.

Primary-progressive (PP) MS. The consensus definition is as follows: disease progression from onset with occasional plateaus and temporary minor improvements allowed (figure 2, a and b).

The essential element in PP-MS is a gradual nearly continuously worsening baseline with minor fluctuations but no distinct relapses. Eighty-one of 131 responses included this definition. Although nearly continuous progression is required, it was recognized that progression at a constant rate throughout disease (figure 2a) was unlikely and that accommodation must be made for variations in the rate of progression over time (figure 2b). A small number of respondents suggested that the definition of PP-MS should include evidence from MRI to distinguish this from other forms of disease (see Discussion).

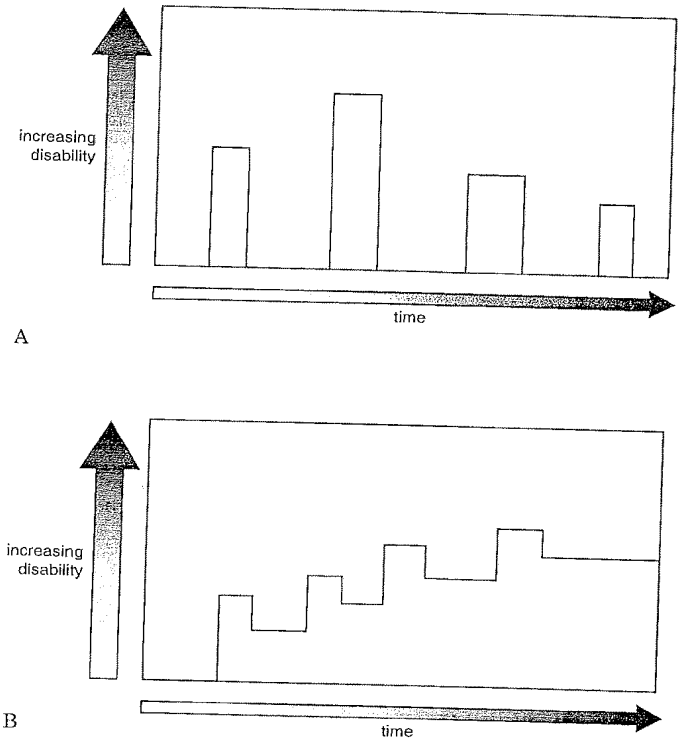


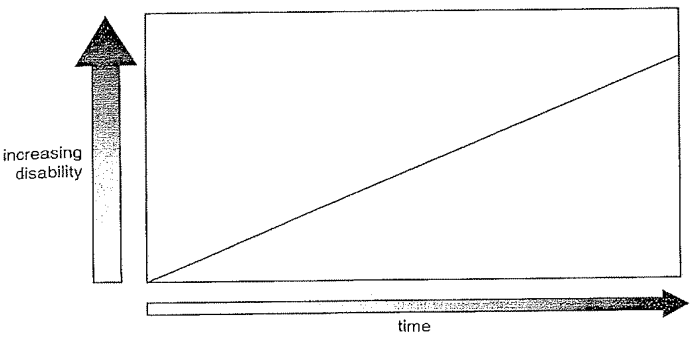
Figure 1. Relapsing-remitting (RR) MS is characterized by clearly defined acute attacks with full recovery (A) or with sequelae and residual deficit upon recovery (B). Periods between disease relapses are characterized by lack of disease progression.

Secondary-progressive (SP) MS. The consensus definition is as follows: initial RR disease course followed by progression with or without occasional relapses, minor remissions, and plateaus (figure 3, a and b).

SP-MS may be seen as a long-term outcome of RR-MS in that most SP patients initially begin with RR disease as defined here. However, once the baseline between relapses begins to progressively worsen, the patient has switched from RR-MS to SP-MS. Eighty-four of 124 respondents chose the above definition.

Relapsing-progressive (RP) MS. There is no consensus definition.

Although this has been one of the most commonly used terms to describe an important clinical form of MS characterized by a combination of relapse and progression, there was no consensus for a definition of RP-MS. Some respondents used this term to describe RR patients who do not fully recover (39/138 responses), which was clearly favored for inclusion in the RR definition (above). Others used this term for those patients who are also defined above as SP (41/138). A smaller group (26/138) indicated that the best definition of this group included patients with disease progression from onset with acute episodes of worsening. Because of the lack of consensus and the overlap of definitions with other categories, we conclude that the term



A

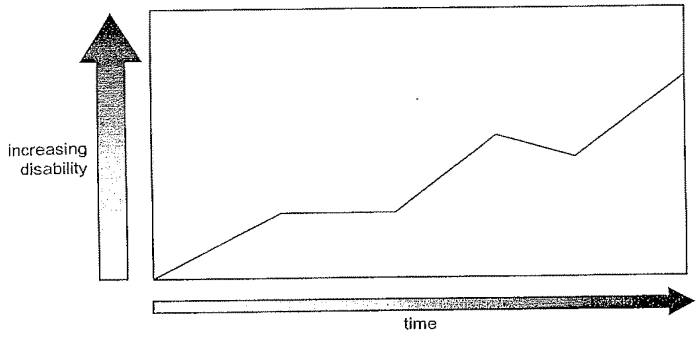


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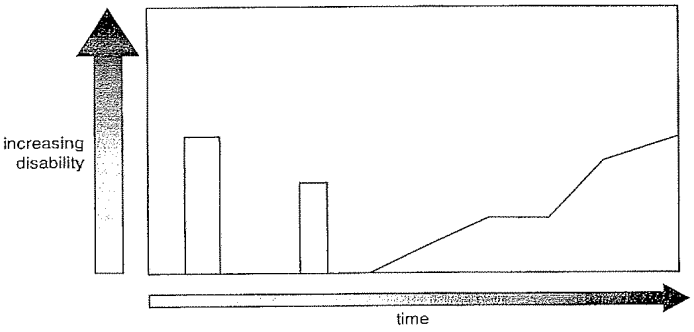
Figure 2. Primary progressive (PP) MS is characterized by disease showing progression of disability from onset, without plateaus or remissions (A) or with occasional plateaus and temporary minor improvements (B).



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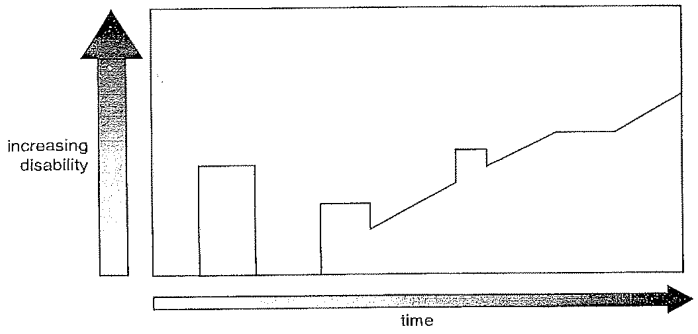

B

Figure 3. Secondary progressive (SP) MS begins with an initial RR course, followed by progression of variable rate (A) that may also include occasional relapses and minor remissions (B).

RP-MS does not correspond to a clearly defined and distinguishable clinical population and should be abandoned.

Progressive-relapsing (PR) MS. The consensus definition is as follows: progressive disease from onset, with clear acute relapses, with or without full recovery; periods between relapses characterized by continuing progression (figure 4, a and b).

Based on the survey and additional discussion, we determined that PR-MS was an additional, albeit rare, clinical course that deserved a separate definition, as it was not included in the other definitions. We propose that this form of MS be termed PR to reflect its progressive onset and to distinguish it from the term RP, for which there was no consensus.

Clinical severity definitions. The above definitions pertain to clinical courses that patients with MS may follow. The survey also queried respondents on two severity outcome definitions, for benign and malignant disease. There was no overwhelming consensus on definitions for these terms and less so for benign disease than malignant disease. Further, many respondents believed that precise definitions were not needed or useful as these terms were not likely to be used as enrollment criteria or end point measures in clinical trials. Although classic definitions have often indicated a set or minimal score on the Kurtzke Expanded Disability Status Scale (EDSS) clinical rating scale,[2,13,14] there was consensus that these should not be used, as they might narrow the clinical picture being described. Further, as these terms do not necessarily reflect future course, it was agreed that they should not be the sole determinant of the appropriateness of any available therapeutic measures. It was additionally emphasized that these terms were most useful in the context of research studies and should be used with care in communication with affected individuals, family members, and third-party payers.

Benign MS. The consensus definition is as follows: disease in which the patient remains fully functional in all neurologic systems 15 years after disease onset.

Malignant MS. The consensus definition is as follows: disease with a rapid progressive course, leading to significant disability in multiple neurologic systems or death in a relatively short time after disease onset.

Discussion. We report here the results of a survey of the international MS clinical research community on terminology commonly used to describe clinical course and outcomes for the disease. We were gratified to find clear preferences and striking agreement on the meaning of the terms RR, PP, and SP forms of MS. Based on survey results and resultant consensus, we added a new term, PR, to represent those patients whose course differs, as diagrammed in figures 1 to 4, from the other definitions. We expect that this represents a small fraction of MS patients.

We found no clear consensus on the definition of

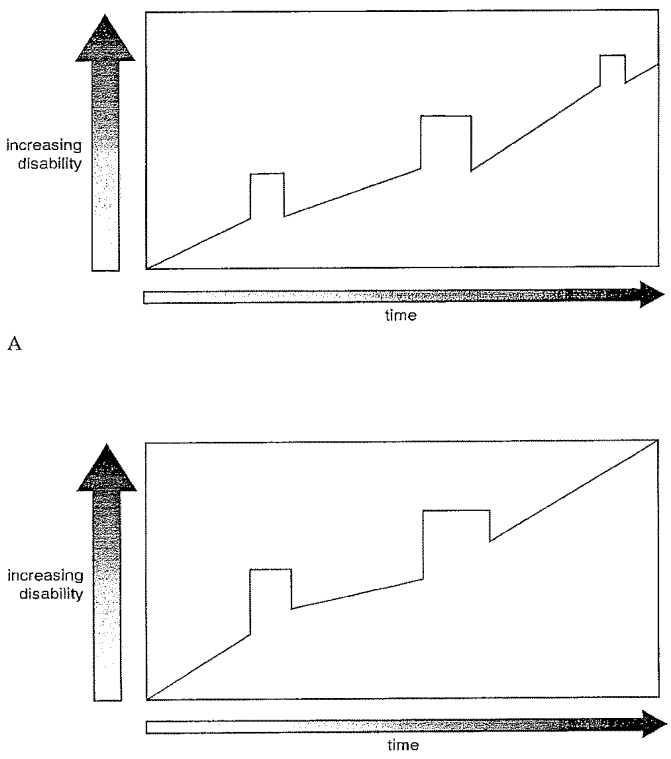

Figure 4. Progressive-relapsing (PR) MS shows progression from onset but with clear acute relapses with (A) or without (B) full recovery.

RP-MS, and because the survey results made it clear that its common usage overlaps with either RR or SP as defined here, we recommend that the term RP-MS be abandoned. Similarly, as the classically used term chronic progressive (CP) MS includes the more recently distinguished groups of PP, SP, and PR patients as newly defined here, we recommend that this term also be abandoned, as being too vague and including forms of MS that differ considerably in clinical course and MRI correlates.[15]

We have not included MRI parameters in these definitions, despite reported differences in MRI lesion load in certain forms of MS (e.g., PP versus SP-MS)[15] because our respondents and committee members with special MRI expertise believed that the current level of knowledge did not allow sufficiently confident association of MS clinical course and MRI findings. This situation could change in the future, as it could for developments relating to any potential biological or surrogate marker of disease activity. If so, we expect the definitions proposed here to be modified accordingly.

We also have not defined a relapse. A relapse implies an acute episode of new disease activity, either a new lesion or fresh activity in an old area of involvement. Both MRI data and neuropathologic studies detail a discordance between the occurrence of MS lesions in the CNS and the development of symptoms or signs.[16-19] A clinical relapse is dependent on involvement of an "eloquent" area of the CNS. Various authors provide definitions. McAlpine defined a relapse as a new symptom or the reappearance of a previous symptom at a time after an initial attack.[1] Schumacher et al.[20] added a requirement for a duration of symptoms of at least 24 hours when evaluating a treatment. For the purposes of a clinical trial, the nature of a relapse will need to be defined by consensus among investigators for each protocol to ensure standardization of the study design. Similarly, for trials of agents being tested for their ability to slow or stop disease progression, duration and rate of progression need to be defined by consensus for inclusion and treatment failure criteria for each trial.

Because at present there are no known clear biological markers that define the various clinical courses of MS, definitions must be made in clinical terms and by consensus among workers in the field. We therefore conclude that the definitions we propose are tenable because they derive from an international survey and input from a large group of MS clinical investigators. Use of these definitions in a standardized fashion will allow more uniformity in clinical descriptions, both in reports in the literature and in designation of patient populations for clinical trials of new agents in MS.

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References

1. Matthews WB, Compston A, Allen IV, Martyn CN, eds. McAlpine's multiple sclerosis. Edinburgh: Churchill Livingstone, 1991.
2. Poser S. Multiple sclerosis: an analysis of 812 cases by means of electronic data processing. Berlin: Springer, 1978.
3. Weinshenker BG, Bass B, Rice GPA, et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain 1989;112:133–146.
4. Phadke JG. Survival patterns and cause of death in patients with multiple sclerosis: results from an epidemiological survey in northeast Scotland. J Neuro Neurosurg Psychiatry 1987;50: 523–531.
5. Confavreux C, Aimard G, Devic M. Course and prognosis of multiple sclerosis assessed by the computerized data processing of 349 patients. Brain 1980;103:281–300.
6. Verjans E, Theys P, Delmotte P, Carton H. Clinical parameters and intrathecal IgG synthesis as prognostic factors in multiple sclerosis. Part I. J Neurol 1983;229:155–165.
7. Confavreux C, Compston DAS, Hommes OR, et al. EDMUS, a European database for multiple sclerosis. J Neuro Neurosurg Psychiatry 1992;55:671–676.
8. The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical

results of a multicenter, randomized, placebo-controlled trial. Neurology 1993;43:655–661.
9. Jacobs LD, Cookfair DL, Rudick RA, et al. Results of a phase III trial of intramuscular recombinant beta interferon as treatment for multiple sclerosis [abstract]. Ann Neurol 1994; 36:259.
10. Johnson KP, Brooks BR, Cohen JA, et al. Copolymer I reduces the relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multi-center, double-blind, placebo-controlled trial. Neurology 1995;45: 1268–1276.
11. Alter M, Byrne TN, Daube JR, et al. Practice advisory on selection of patients with multiple sclerosis for treatment with Betaseron. Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 1994;44: 1537–1540.
12. Whitaker JN, McFarland HF, Rudge P, Reingold SC. Outcomes assessment in multiple sclerosis clinical trials: a critical analysis. Multiple Sclerosis 1995;1:37–47.
13. Kurtzke JF, Beebe GW, Nagler B, et al. Studies on the natural history of multiple sclerosis. 8. Early prognostic features of the later course of the illness. J Chronic Dis 1977;30:819–830.
14. Thompson AJ, Hutchinson M, Brazil J, et al. A clinical and laboratory study of benign multiple sclerosis. Q J Med 1986; 58:69–80.
15. Thompson AJ, Kermode AG, Wicks D, et al. Major differences in the dynamics of primary and secondary progressive multiple sclerosis. Ann Neurol 1991;29:53–62.
16. Isaac C, Li DKB, Genton M, et al. Multiple sclerosis: a serial study using MRI in relapsing patients. Neurology 1988;38: 1511–1515.
17. Miller DH, Rudge P, Johnson G, et al. Serial gadolinium enhanced magnetic resonance imaging in multiple sclerosis. Brain 1988;111:927–929.
18. Koopmans RA, Li DKB, Oger JJF, et al. Chronic progressive multiple sclerosis: serial magnetic resonance brain imaging over six months. Ann Neurol 1989;26:248–256.
19. Willoughby EW, Grochowski E, Li DKB, et al. Serial magnetic resonance scanning in multiple sclerosis: a second prospective study in relapsing patients. Ann Neurol 1989;25:43–49.
20. Schumacher GA, Beebe G, Kibler RF, et al. Problems of experimental trials of therapy in multiple sclerosis. Ann NY Acad Sci 1965;122:552–568.

Multiple sclerosis: recent developments in neuropathology, pathogenesis, magnetic resonance imaging studies and treatment

Claudia Lucchinetti[a], Wolfgang Brück[b] and John Noseworthy[a]

The cause of multiple sclerosis is generally considered to be entirely T cell mediated. However, recent reports of studies in a variety of animal models of inflammatory demyelinating disease, coupled with detailed pathological analysis and neuroimaging studies of multiple sclerosis patients, indicate that the events involved in the formation of the multiple sclerosis lesion may be more complicated. This complex pathogenesis is reflected in the variable response of multiple sclerosis patients to immunomodulatory therapy. Curr Opin Neurol 14:259–269.

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Abbreviations

APL	altered peptide ligands
APP	amyloid precursor protein
CNS	central nervous system
CSF	cerebrospinal fluid
EAE	experimental autoimmune encephalomyelitis
EDSS	expanded disability status score
HHV-6	human herpesvirus type 6
MBP	myelin basic protein
MIP-1α	macrophage inflammatory protein type 1α
MOG	myelin–oligodendrocyte glycoprotein
MRI	magnetic resonance imaging
MS	multiple sclerosis
MTR	magnetization transfer ratio
NAWM	normal-appearing white matter
NO	nitric oxide
PCR	polymerase chain reaction
PPMS	primary progressive multiple sclerosis
RANTES	regulated upon activation: normal T cell expressed/secreted
RRMS	relapsing–remitting multiple sclerosis
SPMS	secondary progressive multiple sclerosis
Th	T helper

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Introduction

The focus of multiple sclerosis (MS) research over the past decade has been on the identification of a single pathogenic mechanism that would allow for the development of targeted therapeutic strategies applicable to all MS patients. Traditionally, MS has been considered to be an autoimmune disorder consisting of myelin autoreactive T cells that drive an inflammatory process, leading to secondary macrophage recruitment, and subsequent myelin destruction [1]. The emphasis on the inflammatory aspects of the MS lesion has been the major impetus for therapeutic strategies to date. This approach has yielded disappointing results. Although inflammatory mechanisms seem to be an important aspect contributing to tissue injury in MS, whether it is a primary or a secondary event in lesion formation is still not clear. Furthermore, the concept that the inflammation in MS is driven by a pure T helper (Th) type 1 cell-mediated response is largely circumstantial, and the potential role of other immune mechanisms must be considered. Accumulating data with increasing numbers of probes that can be effectively applied to MS tissue have indicated that the events involved in the immunopathogenesis of MS may be more complicated. This is not surprising, given the inherent heterogeneity observed with respect to the clinical, radiographic, genetic and morphological features of this disease. This review will focus on recent developments in the field of MS neuropathology, pathogenesis, magnetic resonance imaging (MRI) studies and treatment, and reveal that MS may be more complex than previously recognized.

What drives the multiple sclerosis inflammatory response?

The hallmark of the MS lesion is focal demyelination. These focal areas of myelin destruction occur on a background of an inflammatory reaction consisting of T lymphocytes, a few B lymphocytes and plasma cells, and extensive macrophage/microglial activation. This pathology is similar to that found in experimental autoimmune encephalomyelitis (EAE), an experimental paradigm of Th1 cell-mediated autoimmune disease, and an animal model of MS, which can be induced in susceptible animals by active sensitization with central nervous system (CNS) tissue, myelin, or myelin proteins or by the transfer of autoreactive T cells. MRI studies of early MS suggest that most lesions are preceded by focal blood–brain barrier breakdown. These data have suggested that MS is a primary Th1 cell-mediated disease with secondary macrophage recruitment, and secondary

myelin destruction. In support of this concept, MS is linked to certain MHC class II haplotypes [2]. Autoreactive T cells with a cytokine spectrum of Th1 cells can be isolated from the blood of MS patients, and their frequency appears to be increased in comparison with controls [3]. The inflammatory reaction in MS lesions is associated with the upregulation of a variety of Th1 cytokines, including IL-2, γ-IFN, and TNF-α [4,5], which are also found in the cerebrospinal fluid (CSF) of MS patients with active disease. In addition, the pattern of chemokine expression is also compatible with a Th1-mediated process. Some chemokine receptors, including CCR5 and CXCR3 are overexpressed among lesion-derived T cells and peripheral T cells in patients with MS [6–8]. The α chemokines IP-10 and Mig are predominantly expressed by macrophages and reactive astrocytes within actively demyelinating MS lesions [9•]. Furthermore, MS patients have a significantly increased migratory rate preferentially towards regulated upon activation: normal T cell expressed/secreted (RANTES) and macrophage inflammatory protein type 1α (MIP-1α), but not other chemokines [10•]. The migratory T cell populations represent predominantly Th1/Th0 cells and suggest that the aberrant migration of MS-derived T cells towards RANTES and MIP-1α results from the overexpression of their receptor, CCR5, and can be blocked by anti-CCR5 antibodies. Despite these observations, the evidence supporting a primary and exclusive pathogenic role for Th1-mediated injury in MS remains indirect and circumstantial.

Because Th2 cells antagonize Th1 cell function, many therapeutic efforts in MS have been aimed at down-regulating Th1 by switching responses to Th2. However, therapeutic strategies that are beneficial in EAE have yielded ineffective or at times unexpected aggravation of MS [11]. One possible reason for this discrepancy may be that the pathogenesis of MS lesions is more complex, compared with that of a pure Th1-mediated CNS autoimmune disease. Evidence is accumulating that cells other than the classic Th1 T cells may contribute to the inflammatory process. CD8 class I restricted T cells outnumber CD4 T cells in MS lesions, and predominate at sites of active myelin destruction compared with CD4 T cells, which are mainly restricted to the perivascular region [12]. Recent studies using single-cell polymerase chain reaction (PCR) show that CD8 T cells are clonally expanded compared with the CD4 T cell population [13••]. Axonal destruction in MS lesions correlates better with CD8 T cells and macrophages rather than CD4 T cells [14••].

There is also evidence that Th2 cells can participate in pathological autoimmune processes. MS patients have antibodies to myelin–oligodendrocyte glycoprotein (MOG), and circulating Th2 cells could drive this antibody formation [15]. Antibodies to both MOG and myelin basic protein (MBP) have been demonstrated in MS lesions [16]. Using molecular analyses, several different laboratories have independently shown that B cells and their immunoglobulin products within MS lesions are restricted in diversity [17,18•,19,20,21••]. Genes encoding immunoglobulin within MS plaques were more restricted in gene segment usage than germline DNA, displayed multiple somatic mutations, and had a higher percentage of replacement mutations in the complementary-determining regions than in framework regions. These observations are characteristic of the affinity maturation processes observed in antigen-driven humoral immune responses [21••]. In addition, in-vitro generated Th2 cells from MBP-specific T cell receptor transgenic mice surprisingly caused EAE in immunodeficient mice (both RAG-1 knockout mice and αβ T cell-deficient mice) [22].

Furthermore, the induction of a Th2 effector response can aggravate Th1-mediated autoimmunity. In the marmoset model, EAE can be induced by immunization with MOG, inducing a shift towards a Th2 response that attenuates acute disease, but results in the later onset of a lethal demyelinating disorder [23]. Similar events occurred after attempts to treat MS with a monoclonal antibody to CD52 (a highly glycosylated molecule abundantly expressed by T cells). Therapy with anti-CD52 led to massive T cell depletion, which caused a deviation from a Th1 to a Th2 phenotype, but failed to halt progressive cerebral atrophy or disease progression in MS, despite reducing the relapse rate and MRI activity over an 18-month interval. The switch to Th2 cells also resulted in approximately 40% of cases developing autoimmune thyroiditis or Graves' disease, suggesting that the Th1 cell response may have been converted into a dysregulated Th2 effector cell response [24,25]. These findings underscore the potential danger of therapies based on immune deviation from Th1 cells towards Th2 cells, and suggest that the pathogenesis of MS may be more complex than a pure Th1 cell-mediated CNS autoimmune disease.

Pathogenetic relevance of inflammation in multiple sclerosis

Despite the presence of inflammation in MS, the pathogenic role of the inflammatory response is not clear. There is evidence supporting both the concept that the inflammatory reaction is a prerequisite for demyelination, whereas on the other hand, it may occur independently of demyelination. Neuropathological studies [26–28] revealed that inflammatory cells and specifically T cells are not always present in areas of active demyelination, and persistent inflammation is a frequent and typical feature of chronic MS lesions.

Inflammatory infiltrates are also frequently found in the periplaque white matter and in normal-appearing white matter (NAWM) distant from sites of demyelination. Finally, the abundance of inflammation in inactive lesions, together with recent observations on the local production of neurotrophic factors by leukocytes, may indicate an important role for inflammation in the repair of MS lesions [29].

In addition, the extent of demyelination observed in experimental models of T cell-mediated autoimmune encephalomyelitis is rather limited. This is in contradiction to MS, in which demyelination is the primary feature. Recent magnetic proton spectroscopic data of NAWM suggests that free lipid peaks may be seen in areas not identified as abnormal on T2 or enhanced MRI [30], and in some cases the bulk of T cell inflammation in the lesion apparently follows an initial destruction of myelin [31]. This finding raises the possibility that, at least in some cases, the MS lesion may be initiated within the brain, whereby primary demyelination leads to secondary inflammation. These observations coupled with the limited efficacy of T cell-directed therapies [32,33] suggest that the T cell-mediated immune reaction may not be the only pathogenic immune response involved in MS.

Axonal pathology in multiple sclerosis lesions

Although recent reports have refocused attention on axonal degeneration in MS [34,35], the early literature on MS had already characterized axonal damage in actively demyelinating MS lesions, including the presence of axonal swellings and transections [36•]. Axonal damage occurs within both active and chronic MS plaques. The extent of axonal loss correlates with the reduction of *N*-acetylaspartate in quantitative magnetic resonance spectroscopy, as well as with T1-weighted hypointensity in MRI and with the extent of CNS atrophy in the spinal cord [37–40]. These parameters correlate with clinical disability, and it is therefore thought that axonal damage is a likely cause of chronic disability in MS. The pathogenesis of axonal damage is largely unknown. Furthermore, it is not certain whether demyelination is an essential prerequisite for axonal damage, or whether both conditions appear independently. There is a significant correlation between the reduction in the magnetization transfer ratio (MTR) and the reduction in *N*-acetylaspartate in the MS lesions, especially in patients with secondary progressive multiple sclerosis (SPMS) [41•]. This suggests a tight coupling between damage to myelin and damage to axons. However, reports of as much as a 50% axonal loss in the corpus callosum outside of macroscopic MS lesions suggests that the extensive loss of axons in NAWM may be caused by the effects of axonal transsection of lesions, or the actions of diffusable neurotoxic factors from lesions [42•].

The axonal pathology in chronic inactive MS lesions is typically less pronounced than that seen in active MS lesions. Kornek *et al.* [43••] described in detail the extent of acute axonal injury indicated by amyloid precursor protein (APP) immunoreactivity, in different stages of MS plaque formation. The highest incidence of acute axonal injury was found during active demyelination, which was associated with axonal damage in the periplaque and NAWM of actively demyelinating lesions. In addition, low but significant ongoing axonal injury was observed in inactive demyelinated plaques, but not in completely remyelinated shadow plaques. These findings provide quantitative evidence for a slow burning chronic axonal destruction in completely inactive demyelinated MS plaques, which may ultimately contribute to the clinical progression of the disease. An extensive reduction of axonal density in both the plaques and NAWM within the cervical spinal cord of SPMS patients compared with controls further supports the concept that slow axonal degeneration may contribute to chronic disability in MS [44••].

Most studies on axonal damage have been performed on autopsy cases of patients with either long-standing chronic or fulminant MS. As a result, little is known about axonal damage during the early course of chronic MS. A recent study by Bitsch *et al.* [14••] investigated whether acute axonal injury is linked to active demyelination in MS lesions, by examining brain biopsy specimens from MS patients with early MS. The authors determined that acute axonal injury manifested by the expression of APP was partly independent of demyelination, and could be found in lesions at any stage of demyelinating activity, including remyelination. Axonal damage correlated with the number of macrophages and CD8 cells in the lesions, but not with the expression of TNF-α or inducible nitric oxide (NO) synthase messenger RNA. The study confirmed previous studies indicating that axonal injury in chronic MS may be an early event during the disease course and lesion formation, and suggested that axonal damage could occur independent of active demyelination. Furthermore, the study rendered unlikely the direct involvement of CD4 T lymphocytes, TNF-α and NO as part of the axon-damaging process. A large interindividual variability of axonal pathology, even among MS patients with identical clinical courses, was observed, and suggests that the degree of axonal injury may partly relate to heterogeneous pathogenetic mechanisms of demyelination.

In addition to irreversible axonal injury described in MS, there is also evidence of functional axonal injury. MS

patients often experience rapid clinical fluctuations, not necessarily associated with changes in metabolic status or temperature. The basis for these rapid changes in neurological status is unclear. A study by Brinkmeier *et al.* [45••] suggested that blocking factors may interfere with the axonal conduction. The authors identified a specific pentapeptide (QYNAD) that blocks sodium channels by shifting their steady state inactivation to more negative potentials in NH15-CA2 neuroblastoma × glioma cells. QYNAD was detected at higher concentrations in the CSF of MS patients compared with controls. Given the similarity in the hyperpolarizing shift produced by QYNAD and by local anesthetic agents such as lidocaine, rhey suggested that QYNAD may be an endogenous lidocaine or 'endocaine'. Endocaines may not be the only sodium antagonists that interfere with axonal conduction. Several recent reports indicate that NO can produce reversible conduction block in axons. Demyelinated axons show preferential susceptibility to blockade by NO at concentrations expected at sites of inflammation [46], and there is evidence that this is partly a result of an action of NO on sodium channels [47,48]. However, exposure to higher concentrations of NO can render the block persistent. In addition, even low concentrations of NO can cause persistent conduction block if the axons are concomitantly firing at physiological frequencies, possibly because of the degeneration of the axons at the site of NO exposure. These observations again emphasize that MS may involve more than just damage to myelin. Axons and ion channels may also prove to be potential pathogenetic targets, and need to be considered in the design of future MS therapies.

Oligodendrocyte pathology in multiple sclerosis and implications for remyelination

Demyelination in MS could partly result from the destruction of oligodendrocytes. The fate of the oligodendrocyte in the evolution and repair of the demyelinating lesion is uncertain and is a matter of debate. Oligodendrocyres are susceptible to damage via a number of immune mechanisms present within an inflammatory response. Activared macrophages or microglial cells could mediate oligodendrocyte injury via the production of pro-inflammatory cytokines, such as TNF-α or IFN-γ. TNF-α mRNA expression has been correlated with the appearance of DNA fragmentation in oligodendrocytes within MS lesions [49•]. The expression of TNF-α mRNA in the periplaque white matter negatively correlated with oligodendrocyte numbers. Other oligodendrogliotoxic factors include the generation of reactive oxygen or nitrogen species, the production of excitatory amino acids such as glutamate [50], the activation of complement components, the release of proteolytic and lipolytic enzymes, T cell-mediated injury via T cell products (perforin/lymphotoxin), the interaction of Fas antigen with fas-ligand, CD8 class I MHC-mediated cytotoxicity, or persistent viral infection [51]. Human herpesvirus type 6 (HHV-6) can lead to a pathology mimicking MS [52,53], and appears to localize to oligodendrocytes within MS tissue but not in control tissue [54]. High concentrations of HHV-6 genome detected by in-situ PCR have been reported in MS lesions [55•]. However, the PCR detection of HHV-6 in the CSF of MS patients failed to identify viral DNA in any CSF sample, and antibody titers against HHV-6 were comparable to those seen in the general population, arguing against a causal relationship between HHV-6 and MS [56]. Several studies [57,58] have detected *Chlamydia pneumoniae* in the CSF of most patients with MS, but in only a few controls. However, such studies have not been confirmed in other laboratories [59–61].

Pathological studies have reported a variable degree of oligodendrocyte preservation in actively demyelinating lesions. A recent analysis of oligodendrocyte density relative to lesional activity in a series of 113 MS cases [62•] demonstrated two principal patterns of oligodendrocyte pathology in MS lesions. In the first pattern, oligodendrocytes are variably reduced during the active stages of myelin destruction, but reappear within inactive or remyelinating areas. An increased number of oligodendrocytes in inactive areas expressing proteolipid protein mRNA compared with MOG suggest that these cells may have been derived from the progenitor pool. These cases are characterized by the co-existence of active plaques, demyelinated plaques, and remyelinated shadow plaques. The other pattern is characterized by the extensive destruction of myelinating cells at active sites of demyelination, in the absence of progenitor cell recruitment in inactive plaque areas. In these cases, remyelination is sparse or absent. The profound heterogeneity in the extent and topography of oligodendrocyte desrruction in active demyelinating lesions suggests that, in subsets of MS patients, myelin, mature oligodendrocytes and possibly oligodendrocyre progenitors are differentially affected. The genetic background may also affect the formation of lesions. The apolipoprotein E ε4 allele is associated with more extensive tissue destruction and less efficient repair in MS [63]. These observations suggest that different mechanisms of myelin or oligodendrocyte injury may be operating in individual MS patients.

Remyelination during the early stages of some MS lesions can be extensive. However, it appears to depend on the availability of oligodendrocytes within the lesion. Although the lack of mature oligodendrocytes in the lesion may result in incomplete remyelination, there may be situations in which the stimulation of remyelination might be feasible and useful. The presence of cells in

very early stages of oligodendrocyte development have been identified in completely demyelinated plaques devoid of mature oligodendrocytes [64,65,66•]. To what extent these cells can be stimulated to divide, repopulate the lesions and initiate remyelination must still be demonstrated. In some MS lesions, many cells express low levels of proteolipid protein mRNA in the absence of remyelination. These cells may represent dormant oligodendrocyte progenitors, which may require additional trophic factors to become efficient remyelinating cells. Whether there is a specific radiological correlate for remyelination is questionable. A recent experimental study [67••] observed an increase in MTR during demyelination and remyelination, suggesting that a candidate marker for this process may be available.

Immunopathogenetic heterogeneity

In addition to the heterogeneity observed in the structural aspects of MS lesions, there is an important degree of interindividual variability in the immunopathological features of MS lesions. In a large study of actively demyelinating MS lesions (based on 51 biopsies and 32 autopsies), Lucchinetti *et al.* [68••] recently reported that although most lesions contained an inflammatory reaction mainly composed of lymphocytes and macrophages, quite diverse patterns of myelin destruction were observed. Lesions were analysed using a broad spectrum of immunological and neurobiological markers. The majority of active MS plaques were characterized by the precipitation of immunoglobulins and complement components at sites of active myelin breakdown. These lesions resembled the model of MOG-induced autoimmune encephalomyelitis. However, not all cases of MS followed this pathway. The other cases demonstrated signs suggestive of a primary oligodendrocyte dystrophy. This was reflected either by a disproportionate loss of myelin-associated glycoprotein, and oligodendrocyte apoptosis, or the degeneration of oligodendrocytes in a small rim of periplaque white matter adjacent to active sites of demyelination. These lesions were more reminiscent of virus, ischemic, or toxin-induced demyelination rather than autoimmunity. The patterns of demyelination were heterogeneous between patients, but homogeneous within multiple active plaques from the same patients. Therefore, it is possible that different pathogenic mechanisms of demyelination may operate in different subgroups of MS patients. If this can be confirmed, the identification of paraclinical markers of the underlying pathological processes might guide the appropriate treatment regimen for individual patients.

Magnetic resonance imaging and multiple sclerosis

MRI is not only used for the diagnosis of MS, but has become increasingly important in order to monitor disease activity, to establish prognostic parameters, and to find the radiological correlate of key morphological features of the MS lesion, such as inflammation, demyelination, remyelination or axonal loss [69–71]. There is a poor relationship between magnetic resonance measurements and clinical findings, and T2-weighted images in particular lack pathological specificity. Therefore, the main purpose of current MRI research focuses on the establishment of new magnetic resonance measurements that might correlate better with disability or clinical course, and reflect specific pathological events occurring in either the MS plaque or in NAWM.

General radiological markers include total lesion load (T1 or T2), T1 hypointense lesion load and gadolinium enhancement. A recent retrospective analysis [72••] identified the open-ring imaging sign as highly specific for demyelinating lesions. This phenomenon was associated with macrophage infiltration in a previous study [40]. Gadolinium-enhancing lesions on MRI reflect increased permeability of the blood–brain barrier and inflammation within the lesions [40]. The number of perforin mRNA-expressing cells in the CSF was shown to correlate with gadolinium-enhancing lesions in MS patients [73]. This suggests a possible pathogenetic role for perforin in the destruction of the blood–brain barrier. The immune system may also have implications for the development of T2 lesion volume. When compared on the basis of T2 lesion volume, primary progressive multiple sclerosis (PPMS) patients with a high lesion volume have a higher in-vitro migration and IFN-γ production by the T cells [74••].

Different magnetic resonance parameters have been studied for their relevance and their correlation to clinical disability in MS patients. A serial MRI study [75] revealed an increase in lesion volume over 1 year in all patient groups, except in those who were clinically stable. The increase in expanded disability status score (EDSS) and the occurrence of attacks correlated with the number of gadolinium-enhancing lesions in the course of relapsing-remitting disease [75]. Magnetization transfer has become an important tool for the characterization of MS patients. The MTR is significantly lower in MS patients [76], and there may be differences in MTR between patients with different disease courses. SPMS patients have a lower MTR than relapsing-remitting patients [77]. The correlation of MTR with clinical disability is controversial, with reports ranging from a decrease in the MTR independent of the EDSS [78] to a weak [79] or significant correlation between the MTR [80] and clinical disability. These differences may partly be due to the fact that different regions, such as the whole brain or spinal cord, were studied.

Diffusion imaging may be another method to define the radiological substrate of brain damage more effectively. The average apparent diffusion coefficient was shown to be elevated in MS patients, especially those with a secondary progressive disease course [81,82].

Axon pathology in MS is considered to be an important feature of MS plaques, and may represent the substrate of disability [83]. T1 hypointense lesions are thought to represent axonal damage in MS lesions and to correlate with clinical disability [84]. A comparative pathological–radiological study in postmortem autopsy cases showed that MTR and T1 contrast ratio significantly correlated with axonal density in the lesions [85•]. These hypointense T1 lesions revealed significantly lower concentrations of *N*-acetylaspartate in proton magnetic resonance spectroscopy [86••]. *N*-acetylaspartate is regarded as a biochemical marker of axon integrity, and therefore is considered to be the most reliable current marker for monitoring axonal pathology *in vivo* [87]. The relationship between *N*-acetylaspartate levels and MTR is uncertain. A correlation between *N*-acetylaspartate loss and MTR has been reported in SPMS patients [41•]. However, another study of stable MS patients [88••] did not find a correlation between these parameters, suggesting that demyelination might occur independent of axonal damage. This conclusion was also supported by a detailed morphological study [14••]. Nonetheless, if carefully interpreted, *N*-acetylaspartate may be an attractive candidate molecule to monitor different features of the disease *in vivo*, including disease load, functional axonal recovery and differences in axonal pathology in different disease subgroups [89••,90–92].

Axonal damage may eventually lead to the loss of brain tissue and brain atrophy, a potential new surrogate marker of MS [93]. Progressive cerebral atrophy was shown to be an important feature of the disease [94••], and is more pronounced in patients with an increase in their EDSS [95]. The correlation between brain atrophy and EDSS is stronger in SPMS versus relapsing–remitting multiple sclerosis (RRMS) [96•]. Brain atrophy is thought to be an early event in the development of the disease [97•], and is closely associated with the T1 hypointense lesion load [98].

Cognitive deficits are frequently observed in MS patients; however, the pathological substrate of this feature is not yet clear. The cortical/subcortical disease burden in MS patients seems to have an important impact on the development of cognitive impairment [99•,100,101] and on cortical metabolism [102•]. The total lesion load, in contrast, is not well correlated with cognitive function in MS patients [103,104].

Pathological changes in NAWM may also contribute to clinical symptoms, including cognitive impairment, as reflected by decreases in NAWM MTR [105••] or disability, as shown by low *N*-acetylaspartate levels in NAWM [106••]. Patients with either RRMS, PPMS or SPMS have lower MTR than control subjects [107•]. The pathogenesis of changes in NAWM are not yet clear. Axonal injury in a demyelinating lesion may lead to Wallerian degeneration of axons in the surrounding white matter, and may thus predispose to lesion development [108]. On the other hand, changes in tissue integrity may precede lesion formation, indicating an intrinsic process in the CNS being responsible for lesion development. Although it has generally been accepted that the breakdown of the blood–brain barrier is the initial event in lesion formation, a detailed and careful diffusion MRI study [109••] suggested the opposite, namely that subtle changes in NAWM may precede and perhaps trigger the formation of demyelinated plaques. These observations are in agreement with the heterogeneous pathology of MS plaques observed in recent pathological studies [62•,68••].

Multiple sclerosis therapy

The major goal of identifying the mechanisms of injury in the MS lesion is to design effective therapies that are safe and long lasting. The focus on the inflammatory aspects of the MS lesion has been the major impetus for therapeutic strategies to date. This is largely driven by the ease of demonstrating inflammation-mediated neurological injury after autoantigen immunization or the adoptive transfer of CNS-reactive T cells in experimental models of MS. Unfortunately, this approach has been limited in predicting effective therapies for immune-mediated demyelination in humans. The results of several recently completed MS clinical trials are discussed.

Interferon β-1a in patients at risk of multiple sclerosis

The phase III randomized, placebo-controlled trial of 383 patients considered at high risk of developing clinically definite MS (clinically isolated syndrome with baseline MRI evidence of at least two asymptomatic lesions suggestive of MS) [110••] reported an apparent treatment advantage from the administration of IFN-β-1a. 'At risk' patients received corticosteroids and then either active or placebo treatment, and were followed at 6-monthly intervals until they developed either a relapse or worsening, meeting criteria for clinically definite MS in the opinion of an independent, blinded committee. Fifty per cent of the placebo group 'converted' to MS compared with 35% of the IFN-treated patients. A treatment advantage in MRI activity was also apparent in the patients who remained well at the time the trial was stopped after a planned interim analysis. The duration of the study was shorter than most trials (55% followed for

2 years, 34% for 3 years). The findings provide additional support for the theory that beta interferons favorably influence presumed inflammatory-demyelinating disease activity, and are not unexpected. However, the investigators have not so far reported the clinical findings (e.g. stability of the baseline or follow-up neurological findings). The decision to remove patients from the study upon conversion to definite MS precluded the opportunity to measure and report treatment effects in the patients who had a second event (in total 192/383; 50%). These findings may change practice patterns in some settings. Those considering treating all such 'at risk' patients should be mindful that no published study has demonstrated a long-term benefit (e.g. beyond 4 years) in the development of clinical disability. In addition, there are healthcare costs and adverse effect issues, and the difference between those 'fortunate' to be randomly assigned to active drug and those who received placebo are less convincing when one considers the combined outcome of 'discontinued treatment early' or 'conversion to MS' (44% IFN versus 54% placebo).

Glatiramer acetate

The investigators of the North American study of glatiramer acetate in RRMS [111•] reported their experience with a 6-year extension study of 208 of the original 251 patients. The finding that treated RRMS patients showed less disability progression than predicted from natural history information needs to be considered with caution, however. Observational studies require both complete follow-up and the appropriate use of natural history information. Unfortunately, this was not the case with their report, in that 27% of patients who joined the extension study were lost to follow-up, and the natural history database (historical controls) included patients with both relapsing and progressive MS. In a second study [112•], glatiramer acetate was shown to reduce MRI evidence of ongoing disease activity in 27 RRMS patients, providing support to a previous report [113].

Cladribine in progressive multiple sclerosis

Cladribine (2-chlorodeoxyadenosine) reduced MRI evidence of disease progression (enhanced T1 lesion number and volume; T2 lesion load) compared with placebo in a randomized, placebo-controlled, variable dose trial in 159 patients with PPMS and SPMS [114•]. The MRI effect was seen primarily in the SPMS patients. The effect on the T2 lesion load was seen with the higher dose only. The duration of the trial was too short (one year) to have any chance of demonstrating a clinical benefit using EDSS change, regrettably.

Does intravenous immunoglobulin enhance recovery in multiple sclerosis?

A non-randomized, placebo-controlled crossover trial of intravenous immunoglobulin [115] failed to demonstrate improvements in central motor conduction time in 10 RRMS patients. In a randomized, double-blind, placebo-controlled study [116], intravenous immunoglobulin was not shown to reverse long-standing motor deficits in 67 patients with established weakness.

Antigen-specific immunotherapy

Two phase II trials of specific immunotherapy designed to block T cell responses in RRMS using altered peptide ligands (APL) of myelin antigens were reported in 2000. The APL administered in the first trial (CGP77116) was poorly tolerated (continued relapses, systemic hypersensitivity reactions) despite dose reduction, and was terminated when only eight of the planned 24 patients had been enrolled [117]. Two of the three patients with on-study relapses developed high precursor frequencies of T cells reacting to both the APL and $MBP_{(83-89)}$, suggesting that treatment may have led to increased disease activity. The second trial was also terminated for safety reasons when hypersensitivity reactions developed in 9% of the 142 enrolled patients [118]. Immunological studies demonstrated that treatment was followed by regulatory Th2 responses to APL and MBP in the study. There was MRI evidence that the low dose of the APL in the study (NBI5788, 5 mg subcutaneously once a week; dose range tested in trial: placebo, 5, 20 and 50 mg) was followed by reduced subclinical disease activity, but the short trial duration prevented any evaluation of meaningful clinical efficacy. Such studies illustrated both the potential for benefit and harm with immunotherapy, and further work is in progress with these treatment approaches.

The intravenous administration of different doses of a solubilized complex of human leukocyte antigen-specific DR2 with MBP^{84-102} (AG284) was safe, but was without evidence of clinical or MRI effect or induced T cell tolerance to MBP or MBP^{84-102} in 33 patients with SPMS [119].

Conclusion

The hallmark of the MS lesion is multifocal demyelination. The clinical deficits are partly dependent on demyelination, but also on subsequent axonal injury. The extent to which demyelination and axonal injury in MS are a direct consequence of inflammation is still uncertain. Apart from focusing on the inflammatory aspects of the MS lesion in the design of therapeutic strategies, novel approaches to inhibit demyelination, prevent neuronal death, protect axons or promote remyelination will probably be required for effective therapy. Future studies will also need to define specific clinical or paraclinical parameters that allow for differentiating the heterogeneous pathogenetic components in MS lesions, in order to tailor current or future MS therapies more effectively.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Wekerle H. Immunology of multiple sclerosis. In: McAlpine's multiple sclerosis, 3rd ed. Compston A (editor). London: Churchill Livingstone; 1998.

2 Kalman B, Lublin FD. The genetic of multiple sclerosis. A review. Biomed Pharmacother 1999; 53:358–370.

3 Merrill JE. Proinflammatory and antiinflammatory cytokines in multiple sclerosis and central nervous system acquired immunodeficiency syndrome. J Immunother 1992; 12:167–170.

4 Cannella B, Raine CS. The adhesion molecule and cytokine profile of MS lesions. Ann Neurol 1995; 37:424–435.

5 Navikas V, Link H. Cytokines and the pathogenesis of multiple sclerosis. J Neurosci Res 1996; 45:322–333.

6 Balazhov KE, Rottman JB, Weiner HL, *et al.* CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. Proc Natl Acad Sci USA 1999; 96:6873–6878.

7 Sorenson TL, Tani M, Jensen J, *et al.* Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. J Clin Invest 1999; 103:807–815.

8 Strunk T, Bubel S, Mascher B, *et al.* Increased numbers of CCR5+ interferon-gamma and tumor necrosis factor-alpha-secreting T lymphocytes in multiple sclerosis patients. Ann Neurol 2000; 47:269–273.

9 • Simpson JE, Newcombe J, Cuzner ML, *et al.* Expression of the interferon-γ-inducible chemokines IP-10 and Mig and their receptor, CXCR3, in multiple sclerosis lesions. Neuropathol Appl Neurobiol 2000; 26:133–142.
This study provides new information about the role of specific alpha chemokines and their receptors in MS pathology pathogenesis.

10 • Zang YCQ, Samata AK, Halder JB, *et al.* Aberrant T cell migration toward RANTES and MIP-1alpha in patients with multiple sclerosis: overexpression of chemokine receptor CCR5. Brain 2000; 123:1874–1882.
This study uses functional assays to identify the chemokines associated with aberrant T cell trafficking in MS, and describes for the first time that peripheral T cells obtained from MS patients exhibit a unique migratory pattern toward RANTES and MIP-1α.

11 Hohlfeld R. Biotechnological agents for the immunotherapy of multiple sclerosis: principles, problems and perspectives. Brain 1997; 120:865–916.

12 Booss J, Esiri MM, Tourtellotte WW, *et al.* Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. J Neurol Sci 1983; 62:219–232.

13 •• Babbe H, Roers A, Waisman A, *et al.* Clonal expansion of CD8 T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. J Exp Med 2000; 192:393–404.
Using a PCR-based technique, this study determined the clonal composition and T cell receptor repertoire of CD4 and CD8 T cell infiltrates in actively demyelinating MS lesions with unprecedented resolution at the level of single cells. V region sequence analysis revealed the presence of large clonal expansions primarily among CD8 T cells.

14 •• Bitsch A, Schuchardt J, Bunkowski S, *et al.* Acute axonal injury in multiple sclerosis: correlation with demyelination and inflammation. Brain 2000; 123:1174–1183.
This detailed morphological study correlated APP immunostaining to demyelinating activity in MS lesions, and concluded that axonal injury may partly occur independent of demyelinating activity. In addition, APP expression in damaged axons correlated with the numbers of macrophages and CD8 T lymphocytes within MS lesions.

15 Lindert RB, Haase CG, Brehm U, *et al.* Multiple sclerosis: B- and T-cell responses to the extracellular domain of the myelin oligodendrocyte glycoprotein. Brain 1999; 122:2089–2100.

16 Genain CP, Cannella B, Hauser SL, *et al.* Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat Med 1999; 5:170–175.

17 Branzini SE, Jeong MC, Butunoi C, *et al.* B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions. J Immunol 1999; 163:5133–5144.

18 • Colombo E, Dono M, Gazzola P, *et al.* Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients. J Immunol 2000; 164:2782–2789.
This study used PCR methodology to demonstrate a preferential expansion of certain B cell clones in MS patients.

19 Owens GP, Kraus H, Burgoon MP, *et al.* Restricted use of VH4 germline segments in acute MS brain. Ann Neurol 1998; 43:236–243.

20 Qin Y, Duquette P, Zhang Y, *et al.* Clonal expansion and somatic hypermutation of Vh genes of B cells from cerebrospinal fluid in multiple sclerosis. J Clin Invest 1998; 102:1045–1050.

21 •• Smith-Jensen T, Burgoon MP, Anthony J, *et al.* Comparison of IgG heavy chain sequences in MS and SSPE brain reveals an antigen-driven response. Neurology 2000; 54:1227–1232.
This study used PCR technology to analyse the IgG heavy chain variable region repertoire found in MS brains and compared it with brain VH sequences in patients with SSPE. Similar to SSPE, MS VH sequences were mutated extensively, displayed a preferential accumulation of replacement mutations in complementary-determining regions, and were unique in each brain, findings compatible with an antigen-driven response in MS.

22 Lafaille JJ, Keere FV, Hsu AL, *et al.* Myelin basic protein specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. J Exp Med 1997; 186:307–312.

23 Genain CP, Nguyen MH, Letvin NL, *et al.* Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. J Clin Invest 1995; 96:2966–2974.

24 Coles AJ, Wing MG, Molyneux P, *et al.* Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. Ann Neurol 1999; 46:296–304.

25 Coles AJ, Wing M, Smith S, *et al.* Pulsed monoclonal antibody treatment and autoimmune thryoid disease in multiple sclerosis. Lancet 1999; 354:1691–1695.

26 Dawson JW. The histology of disseminated sclerosis. Edin Med J 1916; NS17:311–344.

27 Guseo A, Jellinger K. The significance of perivascular infiltrations in multiple sclerosis. J Neurol 1975; 211:51–60.

28 Rodriguez M, Scheithauer BW. Ultrastructure of multiple sclerosis. Ultrastructural Pathol 1994; 18:3–13.

29 Kerschensteiner M, Gallmeier E, Behrens L, *et al.* Activated human T cells, B cells and monocytes produce brain-derived neurotrophic factor (BDNF) *in vitro* and in brain lesions: a neuroprotective role for inflammation? J Exp Med 1999; 189:865–870.

30 McFarland HF. The lesion in multiple sclerosis: clinical, pathological, and magnetic resonance imaging considerations. J Neurol Neurosurg Psych 1998; 64 (suppl.):S26–S30.

31 Gay FW, Drye TJ, Dick GW, Esiri MM. The application of multifactorial cluster analysis in the staging of plaques in early multiple sclerosis. Identification and characterization of primary demyelinating lesion. Brain 1997; 120:1461–1483.

32 Linsey JW, Hodgkinson S, Mehta R, *et al.* Repeated treatment with chimeric anti-CD4 antibody in multiple sclerosis. Ann Neurol 1994; 36:183–189.

33 Barkhof F, Thompson AJ, Hodgkinson S, *et al.* Double-blind placebo-controlled MR monitored exploratory trial of chimeric anti-CD4 antibodies in NS [Abstract]. J Neuroimmunol 1995; (Suppl.) 1:15.

34 Trapp BD, Peterson J, Ransohoff RM, *et al.* Axonal transsection in the lesions of multiple sclerosis. N Engl J Med 1998; 338:278–285.

35 Ferguson B, Matyszak MK, Esiri MM, *et al.* Axonal damage in acute multiple sclerosis. Brain 1997; 120:393–399.

36 • Kornek B, Lassmann H. Axonal pathology in multiple sclerosis: a historical note. Brain Pathol 1999; 9:651–656.
This paper gives a full account of the historical literature on axonal pathology in MS.

37 Barnes D, Munro PMG, Youl BD, *et al.* The longstanding MS lesion. A quantitative MRI and electron microscopic study. Brain 1991; 114:1271–1280.

38 Losseff NA, Webb SL, O'Riordan JI, *et al.* Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. Brain 1996; 119:701–708.

39 van Walderveen MAA, Kamphorst W, Scheltens P, *et al.* Histopathologic correlate of hypointense lesions on T1-weighted spin-echo MRI in multiple sclerosis. Neurology 1998; 50:1282–1288.

40 Brück W, Bitsch A, Kolenda H, *et al.* Inflammatory central nervous system demyelination: correlation of magnetic resonance imaging findings with lesion pathology. Ann Neurol 1997; 42:783–793.

41 • Davie CA, Silver NC, Barker GJ, *et al.* Does the extent of axonal loss and demyelination from chronic lesions in multiple sclerosis correlate with clinical subgroup? J Neurol Neurosurg Psychiatry 1999; 67:710–715.
This study used magnetic resonance spectroscopy and magnetization transfer imaging on 18 MS patients and concluded that axonal loss in MS lesions correlated with the extent of demyelination.

42 • Evangelou N, Asiri M, Smith S, *et al.* Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. Ann Neurol 2000; 47:391–395.
This study quantitated axonal loss in NAWM of the corpus callosum in postmortem MS brains, and confirmed that substantial axonal loss occurs in the NAWM.

43 •• Kornek B, Storch M, Weissert R *et al.* Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive and remyelinated lesions. Am J Pathol 2000; 157:267–276.
This study describes in detail the extent of acute axonal injury (using APP) in different stages of MS plaque formation. It also gives for the first time quantitative evidence for a slow burning chronic axonal destruction in completely inactive demyelinated, but not in remyelinated plaques.

44 •• Lovas G, Szilagyi N, Majtenyi K, *et al.* Axonal changes in chronic demyelinated cervical spinal cord plaques. Brain 2000; 123:308–317.
This study used neurofilament staining to estimate axonal loss quantitatively in the cervical spinal cord of patients with SPMS, and observed a significant reduction of axonal density both in the plaque and NAWM compared with control cases. These observations support the concept of a slow axonal degeneration, rather than acute damage, as a cause of chronic disability in MS.

45 •• Brinkmeier H, Aulkemeyer P, Wollinsky KH, *et al.* An endogenous pentapeptide acting as a sodium channel blocker in inflammatory autoimmune disorders of the central nervous system. Nat Med 2000; 6:808–811.
This study describes the characterization of a putative sodium antagonist (QYNAD) in the CSF of MS patients, which may play an important role in the pathophysiology of fluctuating MS symptoms, as well as providing a potential target for future MS therapies.

46 Redford EJ, Hall SM, Smith KJ. Vascular changes and demyelination induced by the intraneural injection of tumor necrosis factor. Brain 1995; 118:869–878.

47 Renganathan M, Cummins TR, Hormuzdiar WN, *et al.* Nitric oxide is an autocrine regulator of Na+ currents in axotomized C-type DRG neurons. J Neurophysiol 2000; 83:2431–2443.

48 Kapoor R, Davies M, Smith KJ. Temporary conduction block and axonal loss in inflammatory neurologic disease. Ann NY Acad Sci 1999; 893:304–308.

49 • Bitsch A, Kuhlmann T, Da Costa C, *et al.* Tumour necrosis factor alpha mRNA expression in early multiple sclerosis lesions: correlation with demyelinating activity and oligodendrocyte pathology. Glia 2000; 29:366–375.
This detailed pathological study examined TNF-α expression with respect to demyelinating activity in MS lesions, and found that TNF-α mRNA negatively correlated with oligodendrocyte numbers, suggesting a possible pathogenetic role for TNF-α in MS.

50 Pitt D, Werner P, Raine C. Glutamate excitotoxicity in a model of multiple sclerosis. Nat Med 2000; 6:67–69.

51 Lucchinetti CF, Bruck W, Lassmann H. Multiple sclerosis: lessons learned from neuropathology. Semin Neurol 1998; 18:337–349.

52 Harrington D, Knox KK. Subacute leukoencephalitis caused by CNS infection with human herpesvirus-6 manifesting as acute multiple sclerosis. Neurology 1996; 47:145–148.

53 Sanders VJ, Waddell AE, Felisan SL, *et al.* Herpes simplex virus in postmortem multiple sclerosis brain tissue. Arch Neurol 1996; 53:123–124.

54 Challoner PB, Smith KT, Parker JD, *et al.* Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. Proc Natl Acad Sci USA 1995; 92:7440–7444.

55 • Blumberg BM, Mock DJ, Powers JM, *et al.* The HHV6 paradox: ubiquitous commensal or insidious pathogen? A two-step in situ PCR approach. J Clin Virol 2000; 16:159–178.
This study utilized a two-step in-situ PCR approach to examine for the presence of HHV-6 in MS and progressive multifocal leukoencephalopathy brain tissue, and found high concentrations of HHV-6 genome in association with both MS and progressive multifocal leukoencephalopathy lesions, suggesting that HHV-6 activation may play a role in the pathogenesis of these two diseases.

56 Taus C, Pucci E, Cartechini E, *et al.* Absence of HHV-6 and HHV-7 in cerebrospinal fluid in relapsing–remitting multiple sclerosis. Acta Neurol Scand 2000; 101:224–228.

57 Sriram S, Stratton CW, Yao S, *et al. Chlamydia pneumoniae* infection of the central nervous system in multiple sclerosis. Ann Neurol 1999; 46:6–14.

58 Leyh-Schmitt G, Bendl C, Hildt U. Evidence for infection with *Chlamydia pneumoniae* in a subgroup of patients with multiple sclerosis. Ann Neurol 2000; 47:652–655.

59 Boman J, Roblin PM, Sundstrom P, *et al.* Failure to detect *Chlamydia pneumoniae* in the central nervous system of patients with MS. Neurology 2000; 54:265.

60 Morre S, De Groot CJA, Killestein J, *et al.* Is *Chlamydia pneumoniae* present in the central nervous system of multiple sclerosis patients. Ann Neurol 2000; 48:399.

61 Pucci E, Taus C, Cartechini E, *et al.* Lack of *Chlamydia* infection of the central nervous system in multiple sclerosis. Ann Neurol 2000; 8:399–400.

62 • Lucchinetti C, Bruck W, Parisi J, *et al.* A quantitative analysis of oligodendrocytes in multiple sclerosis lesions: a study of 113 cases. Brain 1999; 122:2279–2295.
This study quantitatively examined the fate of oligodendrocytes with respect to demyelinating activity in the largest series of MS lesions reported. A profound heterogeneity in the extent and topography of oligodendrocyte destruction in active MS lesions suggested that in subsets of MS patients, myelin, mature oligodendrocytes, and possibly oligodendrocyte progenitors may be differentially affected.

63 Fazekas F, Strasser-Fuchs S, Schmidt H, *et al.* Apolipoprotein E genotype related differences in brain lesions of multiple sclerosis. J Neurol Neurosurg Psychiatry 2000; 69:25–28.

64 Archelos JJ, Trotter J, Previtali S, *et al.* Isolation and characterization of an oligodendrocyte precursor-derived B-cell epitope in multiple sclerosis [see Comments]. Ann Neurol 1998; 43:15–24. Comment in Ann Neurol 1998; 43:4–6.

65 Wolswijk G. Oligodendrocyte precursor cells in chronic multiple sclerosis lesions. Multiple Sclerosis 1997; 3:168–169.

66 • Chang A, Nishiyama A, Peterson J, *et al.* NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. J Neurosci 2000; 20:6404–6412.
This study identified cells with phenotypic markers of endogenous oligodendrocyte progenitors in the mature human CNS, and suggests that functional subpopulations of NG2-positive cells exist in MS lesions, and these cells may represent a viable target for future therapies designed to promote remyelination in MS patients.

67 •• Deloire-Grassin MSA, Brochet B, Quesson B, *et al.* In vivo evaluation of remyelination in rat brain by magnetization transfer imaging. J Neurol Sci 2000; 178:10–16.
This experimental study provides evidence for magnetization transfer being a candidate marker to visualize demyelination and remyelination *in vivo*.

68 •• Lucchinetti C, Bruck W, Parisi J, *et al.* Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000; 47:707–717.
This study emphasized the heterogeneous nature of MS lesions, and identified four patterns of disease that remain uniform in all active lesions within a given patient. These findings suggest that MS may be a heterogeneous syndrome rather than a unified disease, which has major implications for therapy that may differ radically among patient groups.

69 Castelijns JA, Barkhof F. Magnetic resonance (MR) imaging as a marker for multiple sclerosis. Biomed Pharmacother 1999; 53:351–357.

70 McFarland HF. Correlation between MR and clinical findings of disease activity in multiple sclerosis. Am J Neuroradiol 1999; 20:1777–1778.

71 Rovaris M, Filippi M. Magnetic resonance techniques to monitor disease evolution and treatment trial outcomes in multiple sclerosis. Curr Opin Neurol 1999; 12:337–344.

72 •• Masdeu JC, Quinto C, Olivera C, *et al.* Open-ring imaging sign. Highly specific for atypical brain demyelination. Neurology 2000; 54:1427–1433.
This careful review of pathologically confirmed demyelinating lesions in the literature introduces the open-ring imaging sign as being highly specific for atypical brain demyelination. Although the hypothesis needs further confirmation, it is an attractive approach to identify a demyelination-specific MRI pattern.

73 Kivisäkk P, Stawiarz L, Matusevicius D, *et al.* High numbers of perforin mRNA expressing CSF cells in multiple sclerosis patients with gadolinium-enhancing brain MRI lesions. Acta Neurol Scand 1999; 100:18–24.

74 •• Prat A, Pelletier D, Duquette P, *et al.* Heterogeneity of T-lymphocyte function in primary progressive multiple sclerosis: relation to magnetic resonance imaging volume. Ann Neurol 2000; 47:234–237.
This study tries to establish a relationship between MRI presentation of the disease and immunological parameters in PPMS patients.

75 Weiner HL, Guttmann CRG, Khoury SJ, *et al.* Serial magnetic resonance imaging in multiple sclerosis: correlation with attacks, disability, and disease stage. J Neuroimmunol 2000; 104:164–173.

76 Bozzali M, Rocca MA, Iannucci G, *et al.* Magnetization-transfer histogram analysis of the cervical cord in patients with multiple sclerosis. Am J Neuroradiol 1999; 20:1803–1808.

77 Rovaris M, Bozzali M, Santuccio G, *et al.* Relative contributions of brain and cervical cord pathology to multiple sclerosis disability: a study with magnetisation transfer ratio histogram analysis. J Neurol Neurosurg Psychiatry 2000; 69:723–727.

78 Patel UP, Grossman RI, Phillips MD, *et al.* Serial analysis of magnetization-transfer histograms and expanded disability status scale scores in patients with relapsing–remitting multiple sclerosis. Am J Neuroradiol 1999; 20:1946–1950.

79 Lycklama à Nijeholt GJ, Castelijns JA, Lazeron RHC, *et al.* Magnetization transfer ratio of the spinal cord in multiple sclerosis: relationship to atrophy and neurologic disability. J Neuroimaging 2000; 10:67–72.

80 Iannucci G, Minicucci L, Rodegher M, *et al.* Correlations between clinical and MRI involvement in multiple sclerosis: assessment using T1, T2 and MT histograms. J Neurol Sci 1999; 171:121–129.

81 Nusbaum AO, Tang CY, Wei T-C, *et al.* Whole-brain diffusion MR histograms differ between MS subtypes. Neurology 2000; 54:1421–1426.

82 Scanderbeg AC, Tomaiuolo F, Sabatini U, *et al.* Demyelinating plaques in relapsing–remitting and secondary-progressive multiple sclerosis: assessment with diffusion MR imaging. Am J Neuroradiol 2000; 21:862–868.

83 Trapp BD, Ransohoff R, Rudick R. Axonal pathology in multiple sclerosis: relationship to neurologic disability. Curr Opin Neurol 1999; 12:295–302.

84 Truyen L, van Waesberghe JHTM, van Walderveen MAA, *et al.* Accumulation of hypointense lesions ('black holes') on T1 spin-echo MRI correlates with disease progression in multiple sclerosis. Neurology 1996; 47:1469–1476.

85 • van Waesberghe JHTM, Kamphorst W, De Groot CJA, *et al.* Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Ann Neurol 1999; 46:747–754.
This study compared axon loss in chronic, autopsy-derived MS lesions with MTR and T1 contrast ratio and found a strong correlation between these parameters indicating that axon loss contributes significantly to MTR and T1 signal.

86 •• Brex PA, Parker GJM, Leary SM, *et al.* Lesion heterogeneity in multiple sclerosis: a study of the relations between appearances on T1 weighted images, T1 relaxation times, and metabolite concentrations. J Neurol Neurosurg Psychiatry 2000; 68:627–632.
This study correlated T1 relaxation times with metabolite concentrations in MS lesions. A relationship was found between prolonged T1 relaxation (hypointense T1 lesions) and lower *N*-acetylaspartate/higher myoinositol concentrations, indicating that T1 hypointensity reflects axonal damage and gliosis.

87 De Stefano N, Narayanan S, Matthews PM, *et al.* Proton MR spectroscopy to assess axonal damage in multiple sclerosis and other white matter diseases. J Neurovirol 2000; 6 (Suppl. 2):S121–S129.

88 •• Pendlebury ST, Lee MA, Blamire AM, *et al.* Correlating magnetic resonance imaging markers of axonal injury and demyelination in motor impairment secondary to stroke and multiple sclerosis. Magn Res Imaging 2000; 18:369–378.
This study correlated MTR and *N*-acetylaspartate loss with motor impairment secondary to stroke and MS. In MS lesions, a dissociation of these two markers was found indicating that demyelination and axon loss may occur independently from each other.

89 •• Gonen O, Catalaa I, Babb JS, *et al.* Total brain *N*-acetylaspartate. A new measure of disease load in MS. Neurology 2000; 54:15–19.
This study introduces total brain *N*-acetylaspartate measurement as a new interesting marker for disease progression and treatment efficacy in MS.

90 Reddy H, Narayanan S, Matthews PM, *et al.* Relating axonal injury to functional recovery in MS. Neurology 2000; 54:236–239.

91 Cucurella M-G, Rovira A, Rio J, *et al.* Proton magnetic resonance spectroscopy in primary and secondary progressive multiple sclerosis. NMR Biomed 2000; 13:57–63.

92 Helms G, Stawiarz L, Kivisäkk P, Link H. Regression analysis of metabolite concentrations estimated from localized proton MR spectra of active and chronic multiple sclerosis lesions. Magn Reson Med 2000; 43:102–110.

93 Jagust WJ, Noseworthy JH. Brain atrophy as a surrogate marker in MS. Faster, simpler, better? Neurology 2000; 54:782–783.

94 •• Fox NC, Jenkins R, Leary SM, *et al.* Progressive cerebral atrophy in MS. A serial study using registered, volumetric MRI. Neurology 2000; 54:807–812.
This important study gives detailed volumetric data on cerebral atrophy in MS, and proves that progressive cerebral volume reduction occurs in a relatively short observation period, and may therefore be an interesting outcome parameter to monitor treatment trials.

95 Inglese M, Rovaris M, Giacomotti L, *et al.* Quantitative brain volumetric analysis from patients with multiple sclerosis: a follow-up study. J Neurol Sci 1999; 171:8–10.

96 • Ge Y, Grossman RI, Udupa JK, *et al.* Brain atrophy in relapsing–remitting multiple sclerosis and secondary progressive multiple sclerosis: longitudinal quantitative analysis. Radiology 2000; 214:665–670.
This study shows that brain atrophy and EDSS correlated better in patients with SPMS than those with relapsing–remitting disease.

97 • Brex PA, Jenkins R, Fox NC, *et al.* Detection of ventricular enlargement in patients at the earliest clinical stage of MS. Neurology 2000; 54:1689–1691.
This study shows that cerebral atrophy as detected by ventricular enlargement occurs in the earliest stages of disease development.

98 Paolillo A, Pozzilli C, Gasperini C, *et al.* Brain atrophy in relapsing–remitting multiple sclerosis: relationship with 'black holes', disease duration and clinical disability. J Neurol Sci 2000; 174:85–91.

99 • Rovaris M, Filippi M, Minicucci L, *et al.* Cortical/subcortical disease burden and cognitive impairment in patients with multiple sclerosis. Am J Neuroradiol 2000; 21:402–408.
This study points out the role of cortical/subcortical disease pathology for the development of cognitive impairment in MS.

100 Moriarty DM, Blackshaw AJ, Talbot PR, *et al.* Memory dysfunction in multiple sclerosis corresponds to juxtacortical lesion load on fast fluid-attenuated inversion-recovery MR images. Am J Neuroradiol 1999; 20:1956–1962.

101 Jeffery DR, Absher J, Pfeiffer FE, Jackson H. Cortical deficits in multiple sclerosis on the basis of subcortical lesions. Multiple Sclerosis 2000; 6:50–55.

102 • Blinkenberg M, Rune K, Jensen CV, *et al.* Cortical cerebral metabolism correlates with MRI lesion load and cognitive dysfunction in MS. Neurology 2000; 54:558–564.
This study finds a correlation between cortical metabolism, MRI lesion load and cognitive dysfunction in MS patients.

103 Comi G, Rovaris M, Falautano M, *et al.* A multiparametric MRI study of frontal lobe dementia in multiple sclerosis. J Neurol Sci 1999; 171:135–144.

104 Fulton JC, Grossman RI, Udupa J, *et al.* MR lesion load and cognitive function in patients with relapsing–remitting multiple sclerosis. Am J Neuroradiol 1999; 20:1951–1955.

105 •• Filippi M, Tortorella C, Rovaris M, *et al.* Changes in normal appearing brain tissue and cognitive impairment in multiple sclerosis. J Neurol Neurosurg Psychiatry 2000; 68:157–161.
This study shows that the pathology occurring in NAWM in MS is a major contributor to cognitive impairment in these patients.

106 •• Lee MA, Blamire AM, Pendlebury S, *et al.* Axonal injury or loss in the internal capsule and motor impairment in multiple sclerosis. Arch Neurol 2000; 57:65–70.
This study demonstrates that axonal pathology, distant from the lesions in NAWM, as determined by *N*-acetylaspartate concentrations, contributes to disability in MS.

107 • Tortorella C, Viti B, Bozzali M, *et al.* A magnetization transfer histogram study of normal-appearing brain tissue in MS. Neurology 2000; 54:186–193.
This study reveals differences in MTR histograms of NAWM between different disease groups and control subjects.

108 Simon JH, Kinkel PR, Jacobs L, *et al.* A wallerian degeneration pattern in patients at risk for MS. Neurology 2000; 54:1155–1160.

109 •• Werring DJ, Brassat D, Droogan AG, *et al.* The pathogenesis of lesions and normal-appearing white matter changes in multiple sclerosis. A serial diffusion MRI study. Brain 2000; 123:1667–1676.
This excellent study provides evidence for heterogeneous lesion development in a serial diffusion MRI study. It suggests that a subtype of MS lesions may have its origin in pathological alterations of the CNS, which precede breakdown of the blood–brain barrier and gadolinium enhancement.

110 •• Jacobs LD, Beck RW, Simon JH, *et al.* Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. CHAMPS Study Group. N Engl J Med 2000; 343:898–904.
This study has received considerable interest in that the results support that IFN-β-1a delays the time to a second relapse if administered to patients at high risk of developing MS. Additional studies are now needed to determine whether early treatment alters the likelihood and severity of later clinical disability.

111 Johnson KP, Brooks BR, Ford CC, *et al.* Sustained clinical benefits of glatiramer
• acetate in relapsing multiple sclerosis patients observed for 6 years. Copolymer 1 Multiple Sclerosis Study Group. Multiple Sclerosis 2000; 6:255–266.
This extension trial adds support to the earlier reports that glatiramer acetate may favorably affect the clinical course of MS, although the evidence that progression is delayed or prevented remains incompletely proved for reasons discussed in the text of this review.

112 Ge Y, Grossman R, Udupa J. Glatiramer acetate (Copaxone) treatment in
• relapsing–remitting MS: quantitative MR assessment. Neurology 2000; 54:813–817.
This study demonstrates that glatiramer acetate has a modest effect on reducing MRI evidence of subclinical disease activity in MS.

113 Comi G, Filippi M, and the Copaxone MRI Study Group. The effect of glatiramer acetate (Copaxone) on disease activity as measured by cerebral MRI in patients with relapsing–remitting multiple sclerosis: a multi-center randomized, double-blind, placebo-controlled study extended by open-label treatment [abstract]. Neurology 1999; 52:A289.

114 Rice GP, Filippi M, Comi G. Cladribine and progressive MS: clinical and MRI
• outcomes of a multicenter controlled trial. Cladribine MRI Study Group. Neurology 2000; 54:1145–1155.
Regrettably, this trial was not designed with sufficient length of follow-up to determine whether progressive MS patients benefit from treatment with this immunosuppressive agent.

115 Stangel M, Boegner F, Klatt CH, *et al.* Placebo controlled pilot trial to study the remyelinating potential of intravenous immunoglobulins in multiple sclerosis. J Neurol, Neurosurg Psychiatry 2000; 68:89–92.

116 Noseworthy JH, O'Brien PC, Weinshenker BG, *et al.* IV immunoglobulin does not reverse established weakness in MS – a double-blind, placebo-controlled trial. Neurology 2000; 55:1135–1143.

117 Bielekova B, Goodwin B, Richert N, *et al.* Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. Nat Med 2000; 6:1167–1175.

118 Kappos L, Comi G, Panitch H, *et al.* Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. Nat Med 2000; 6:1176–1182.

119 Goodkin DE, Shulman M, Winkelhake MJ, *et al.* A phase I trial of solubilized DR2: MBp(84–102) (AG284) in multiple sclerosis. Neurology 2000; 54:1414–1420.

Update on the diagnosis of multiple sclerosis

David H Mattson

The diagnosis of multiple sclerosis has been increasingly standardized over the years and has evolved to incorporate new diagnostic modalities. The gold standard for diagnosing multiple sclerosis remains clinical, with dissemination of typical white matter symptoms and signs in time and space. The Schumacher criteria in 1965 attempted to standardize clinical criteria for diagnosing multiple sclerosis. The Poser criteria in 1983 added evoked potential and cerebrospinal parameters and the McDonald criteria in 2001 added MRI parameters. All criteria for diagnosing multiple sclerosis include the caveat that no alternative diagnosis better explains the clinical picture, making the differential diagnosis of multiple sclerosis critical. Recent availability of first generation immunotherapies for MS has increased pressure to make an early and accurate diagnosis of multiple sclerosis and to use the diagnostic work-up to try to prognosticate a future disease course.

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Multiple sclerosis (MS) is an inflammatory demyelinating presumably autoimmune disease of the CNS white matter [1]. It typically starts between the ages 20 and 40 years, though onset in teenage years or up into the 60s is possible. MS causes symptoms referable to white matter pathways in the brain and spinal cord and in 90% of cases starts as a relapsing–remitting disease (RRMS) [2]. RRMS can be easy to diagnose clinically, when there are discreet typical relapses/attacks/exacerbations/bouts of demyelination accompanied by objective findings on neurological examination, followed by remissions, when symptoms can be absent and neurological examination can be normal. RRMS is more challenging to diagnose clinically when attacks of demyelination are historical and unaccompanied by current objective findings, or where objective findings on exam are minor, mostly sensory (which is by nature subjective), or clouded by functional overlay. The current push to diagnose MS as quickly as possible is also challenging, to be able to start currently available immunotherapies as early as possible and to prevent further brain damage as much as possible. This is where the newer criteria incorporating cerebrospinal fluid (CSF) and MRI parameters, which can be sensitive diagnostically and helpful for prognostication, have particular utility. Half the cases of RRMS, at a median of 10 years from onset, become more insidiously progressive, either in between attacks or independent of attacks [3]. Such cases of secondary progressive MS (SPMS) are generally less difficult to diagnose even by clinical criteria because of the longer history, greater likelihood of finding neurological exam abnormalities and with CSF analysis and MRI being of higher yield. In the current era of first generation partially effective immunotherapies for MS, which appear to be most effective if started as early as possible after an MS diagnosis, it is certainly hoped that virtually all patients can be diagnosed well before they become SPMS. The 10% of MS that starts out with an insidiously progressive course and not attacks, typically with a spastic paraparesis, so-called primary progressive MS (PPMS) and the 10% of PPMS that eventually has superimposed attacks on top of initial progression, so-called progressive relapsing MS (PRMS), present a different diagnostic challenge. Since these

forms of disease do not meet classic clinical criteria for MS, they have been a major focus of the newer diagnostic criteria incorporating especially CSF parameters. The differential diagnosis also broadens in these less typical cases.

Criteria for diagnosing MS have become more complex. Even a quick scan of TABLES 1, 2 and 5, which summarize the generations of diagnostic criteria from the 1960s, 1980s and 2000s, demonstrates the growing complexities of standardized criteria. Whether these complexities add clarity and new insights, or confusion and arbitrary dogma, remains to be seen.

Clinical diagnosis of MS

McAlpine/Schumacher (1965) criteria

The gold standard for diagnosing MS is by clinical criteria and this has been best elucidated by McAlpine and standardized by Schumacher (TABLE 1) [4,5]. These clinical criteria are the foundation for all subsequent criteria for diagnosing MS which incorporate new technologies and MS can and still is diagnosed by purely clinical criteria. The clinical diagnosis of MS requires two temporally dissociated typical attacks of demyelination referable to two geographically separate white matter pathways of the brain or spinal cord, with objective evidence of each lesion. Attacks are typically of somewhat stuttering onset over hours to days and must last more than 24 h unaccompanied by fever. Attacks typically last weeks to months and to be separate attacks, must occur more than a month apart. Attacks of demyelination are often triggered by infectious illnesses, mostly viruses, but usually start as the viral illness is abating. Symptoms and signs which occur in association with fever or overheating and resolve with control of fever or cooling down, represent a 'pseudoexacerbation' and not a real exacerbation. Sudden onset of deficits, especially lasting less than 24 h, suggests transient ischemic attacks.

Typical white matter pathways that are involved in MS attacks include:

- Optic nerves causing painful or painless unilateral more often that bilateral visual loss (optic neuritis, ON)
- Corticospinal tract causing mono-, hemi- or paraparesis (the latter from transverse myelitis, TM)
- Spinothalamic tracts causing mono- or heminumbness, paresthesias, or dysesthesias, or even a sensory level (the latter from TM)
- Medial longitudinal fasciculus causing an internuclear ophthalmoplegia, typically bilateral, causing diplopia, oscillopsia and/or vertigo

Somewhat less common demyelinating involvements, include: facial nucleus or nerve causing facial weakness that can even look peripheral in nature and be diagnosed as a Bell's palsy, trigeminal nerve causing facial numbness or trigeminal neuralgia, vestibular nerve causing vertigo or oscillopsia, cerebellum or brainstem cerebellar pathways causing ataxia of gait or limbs or oscillopsia, or sixth cranial nerve causing diplopia. Lhermitte's phenomena is a classic symptom of MS also seen in other cervical cord diseases, where bending the neck produces electrical shocks down the spine. Neurogenic bowel and bladder involvements and sexual dysfunction can be part of the subacute presentation of TM, but otherwise are more typically part of SPMS and unusual as isolated bouts of demyelination to help make an MS clinical diagnosis. Similarly, cognitive dysfunction is a very unusual presenting complaint for MS. Fatigue is a very typical totally subjective symptom of MS, is unusual as the presenting complaint and often accompanies typical neurological exacerbations. Bouts of new or increased fatigue, unaccompanied by new neurologic symptoms or objective signs, cannot be counted as a typical bout of demyelination to help make an MS diagnosis.

Any first attack of demyelination, especially isolated and partial ON or TM or other brainstem syndrome, but also multifocal demyelination, such as acute disseminated encephalomyelitis (ADEM), represents 'possible' or 'probable' MS. Individuals with ON go on to develop MS in 40–90% of cases, with TM in 10–50% of cases, other isolated typically brainstem events, such as internuclear ophthalmoplegia in 40–60% of cases and ADEM in 15–20% of cases [7–10]. Dense initial episodes of ON or TM or ADEM, with less complete recovery, are more likely to be one-time events and not the beginning of MS. A definite clinical diagnosis of MS cannot be made until a second typical geographically disseminated attack occurs at least 1 month after the initial one.

With recent knowledge from serial MRI studies (discussed below) that clinical attacks of demyelination are the 'tip of the iceberg' of ongoing damage from MS, we are less willing to wait for the second clinical attack of demyelination to occur in order to make a gold standard MS diagnosis and then start an available immunotherapy for clinically definite MS. This is certainly part of the pressure to incorporate more sensitive diagnostic modalities into the MS diagnostic process. If we can gain a feel for the clinical tempo of a person's MS over a significant period of time, it can give us useful prognostic indications. Poor recovery from the first or early attacks, frequent or severe early attacks, early cerebellar attacks and significant disability 2, or especially 5 years from onset of MS, all represent a relatively

Table 1. Clinical criteria for MS diagnosis – McAlpine/Schumacher (from [4–6]).

Clinically definite MS	
	Remitting and relapsing
	≥2 episodes 1 month apart or steadily progressive ≥6 months
	Signs of lesions at two or more sites
	Lesions of predominantly white matter
	Age at onset 10–50 years
	No better explanation
Probable MS	
	Single episode suggestive of MS with signs of multiple lesions at onset
	Good recovery with subsequent variability in signs

poorer prognosis. On the other hand, early mild infrequent attacks with complete recovery, long interval between the first and second MS-defining attack and a benign 2, or especially 5 year course from onset of MS, all are relatively reassuring of a better prognosis [3,11,12].

Any diagnosis of MS must incorporate the caveat that no better diagnosis explains the signs and symptoms, so the section of this article on differential diagnosis is crucial to the MS diagnostic process. Clinical parameters that raise particular concern about an MS diagnosis include: unusually young or old onset, sudden onset attacks, dense attacks, lack of eye findings, lack of bowel or bladder findings, prominent or early mainly cognitive complaints, seizures, peripheral nervous system involvement, prominent family history of similar neurologic problems, or relentless progression from disease onset [13].

Development of more rigorous & standardized diagnostic criteria for MS

Poser (1983) criteria: addition of evoked potential & cerebrospinal fluid parameters

Development of objective CSF and evoked potential tests to help diagnose MS led to the convening of the 'Workshop on the Diagnosis of Multiple Sclerosis', under the chairmanship of Charles Poser [14]. The criteria derived from this workshop have become referred to as the 'Poser criteria' and these criteria were initially used mainly to standardize diagnosis for patients for entry into clinical trials, but over the years also became incorporated into clinical practice (TABLE 2). This workshop embraced the work of Schumacher in using clinical criteria for diagnosing clinically definite MS, in category A1 of TABLE 2. Categories C1 and C2 incorporate the concept of 'probable' MS in situations where one of the two clinical events have no objective evidence for demyelination, or where there is only one event of multifocal demyelination. The comments appended to the Poser criteria are crucial to making an accurate diagnosis of probable or definite clinical or laboratory supported MS, with clinicians occasionally forgetting the caveats about needing multiplicity of events in time and space, whether they be clinical, paraclinical, or CSF.

Categories A2 and C3 and B1 and B3, incorporate so-called 'paraclinical evidence' of demyelination from evoked response testing, as a way to confirm that events referable to visual, brainstem, or somatosensory pathways from the past from which a person has recovered without residual signs, were on the basis of a demyelinating lesion, or to provide objective correlates for current bouts of symptoms without accompanying objective signs on neurological examination [15]. Evoked response testing is the least sensitive of the newer modalities which have been incorporated into the MS diagnostic process, with sensitivity in picking up abnormalities in the relevant visual, somatosensory, or brainstem pathways being 65–85, 40–65, or 15–20% of cases, respectively [15]. If the relevant evoked potential is abnormal, it is very helpful to the MS diagnostic process, but if as often happens, it is normal, it is not helpful at all. The presence of evoked potential abnormalities suggesting demyelinating involvement in a part of the neuraxis different from an initial clinical event can be used as evidence for geographic dissemination of demyelinating disease, but cannot be used as evidence of temporal dissemination, unless a previously normal evoked response test becomes abnormal. In essence, evoked response testing represents a somewhat sensitive and objective extension of the clinical exam.

The Poser criteria also incorporated CSF analysis for detection of an elevated immunoglobulin (Ig)G synthesis in the CNS, or of CSF (but not serum) oligoclonal IgG bands [16]. Elevated CSF IgG levels occur in 60% of MS cases and the CSF IgG index or CNS IgG synthesis rate, which are calculated by formulae that correct for blood–brain barrier breakdown, are elevated in 80–90% of MS cases. Agarose electrophoresis, or more sensitively, isoelectric focusing, detects CSF oligoclonal IgG bands in CSF that are not in serum, in 90–95% of patients with MS. Oligoclonal IgG bands likely reflect chronic antigenic stimulation from an ongoing autoimmune inflammatory process and therefore may be evidence of dissemination of the demyelinating disease process over time. CSF abnormalities are thus incorporated into Poser categories B1, B2 and B3 to make a laboratory supported definite MS diagnosis when there are two clinical episodes only one of which can be collaborated by clinical or paraclinical parameters, or when are multiple lesions, but only one clinical attack. In the latter case, the oligoclonal IgG bands must appear over time. In the comments, B2 and B3 can also be used to diagnose 'the so-called progressive form' of MS, if CSF abnormalities accompany at least 6 months of progression. Early in the course of MS, the CSF oligoclonal IgG bands or elevated IgG index are most likely to be absent, so when one most wants the diagnostic support, it may not be there. Even the presence of oligoclonal IgG bands is fairly nonspecific. CNS infections with many different infectious agents can produce oligoclonal IgG bands, including most of the subacute or chronic infections that are in the differential diagnosis of MS. Oligoclonal IgG bands have also been reported in meningeal carcinomatosis, sarcoidosis and CNS lupus. The presence of oligoclonal IgG bands in the setting of an isolated acute demyelinating event, such as ON or TM, puts a patient at higher risk that this is the first event of what will turn out to be MS, but specific comments in the Poser criteria make it clear that this is not yet laboratory supported definite MS [17,18].

McDonald (2001) criteria: addition of MRI parameters

Introduction of neuroimaging to the MS diagnostic process in the 1980s with CT scanning and the 1990s with MRI scanning, represented a significant improvement in the sensitivity of detection of MS (TABLES 3, 4 & 5). CT scanning with double-dose delayed contrast administration detected MS in about 25% of cases. MRI scanning was a major advancement, increasing sensitivity of detecting MS to around 90%. This led to addendums to the Poser criteria in which a new category of MRI 'paraclinical evidence' was added [19,20]. In addition, studies in which MS patients were monitored by frequent MRI scanning in research protocols revealed that the MS disease process is much more

Table 2. Poser criteria for MS diagnosis (from [14]).

Category		Attacks	Clinical evidence	Paraclinical evidence	CSF OB/IgG[1]
A	Clinically definite[2]				
	CDMS A1	2	2		
	CDMS A2	2	1 and	1	
B	Laboratory-supported[3] definite				
	LSDMS B1	2	1 or	1	+
	LSDMS B2	1	2		+
	LSDMS B3	1	1 and	1	+
C	Clinically probable[4]				
	CPMS C1	2	1		
	CPMS C2	1	2		
	CPMS C3	1	1 and	1	
D	Laboratory-supported[5] probable				
	LSPMS D1	2			+

[1]OB/IgG = oligoclonal bands or increased IgG.
[2]Clinically definite MS (CDMS).
1. Two attacks and clinical evidence of two separate lesions.
2. Two attacks; clinical evidence of one lesion and paraclinical evidence of another, separate lesion.
Comment: The two attacks must involve different parts of the CNS, must be separated by a period of at least 1 month and must each last a minimum of 24 h.
[3]Laboratory-supported definite MS (LSDMS).
The laboratory support consists of demonstration in CSF of IgG oligoclonal IgG bands (OB) or increased CNS synthesis of IgG. Oligoclonal bands must not be present in the patient's serum and the serum IgG level must be normal. This assumes that other conditions causing CSF changes, such as syphilis, subacute sclerosing panencephalitis, sarcoidosis, collagen vascular disease and similar disorders, have been ruled out.
1. Two attacks; either clinical or paraclinical evidence of one lesion; and CSF OB/IgG.
Comment: The two attacks must involve different parts of the CNS and be separated by a minimum of 1 month, each having lasted a minimum of 24 h. One of the episodes must involve a part of the CNS distinct from that demonstrated by the clinical or paraclinical evidence.
2. One attack; clinical evidence of two separate lesions; and CSF OB/IgG.
3. One attack; clinical evidence of one lesion and paraclinical evidence of another, separate lesion; and CSF OB/IgG.
Comment: Historical information cannot be substituted for the clinical evidence. Whether the evidence is clinical or paraclinical, both lesions must not have been present at the time of the first examination and must be separated by at least 1 month. This separation in time is designed to reduce the possibility of including a case of acute disseminated encephalomyelitis. In a patient with the so-called progressive form of MS – without remissions and exacerbations – evidence of clinical pr paraclinical optic nerve involvement, for example, should not have been present at the time the paraparesis first appeared. Under those circumstances and only if steady progression has taken place for at least 6 months, may such a case be accepted as MS.
[4]Clinically probable MS (CPMS).
1. Two attacks and clinical evidence of one lesion.
Comment: The two attacks must involve different parts of the CNS. Historical information cannot be considered as a substitute for the clinical evidence.
2. One attack and clinical evidence of two separate lesions.
3. One attack; clinical evidence of one lesion and paraclinical evidence of another, separate lesion.
[5]Laboratory-supported probable MS (LSPMS).
1. Two attacks and CSF OB/IgG.
Comment: The two attacks must involve different parts of the CNS, must be separated by a minimum of 1 month, and must each have lasted at least 24 h.

active than suspected previously, with five to ten times more MRI activity than clinical activity and with MRI activity being common even when a patient was in clinical remission [21,22]. This suggested the possibility that MS could be diagnosed five to ten times more quickly than previously thought. There was also increasing evidence that MRI provided useful prognostic information about whether individuals experiencing their first clinical attack of demyelination were likely to go on to have MS and that at the time of a diagnosis of MS, the burden of MS disease provided useful prognostic information about future MS disease course [24,25]. This led to a push to incorporate and standardize the way MRI was being used in the MS diagnostic process. The convening of the International Panel on the Diagnosis of MS, chaired by Ian McDonald, led to the development of what are already commonly referred to as the 'McDonald criteria' [23]. These criteria will be most useful and are already being used, to standardize the kind of patients entering into clinical trials, where MS research centers already have standardized MRI protocols and reporting of results. They will not be as useful in general clinical practice until such protocols are generally disseminated and radiologists, neuroradiologists and MRI technicians are trained to use them.

The McDonald criteria built on and incorporated the previous clinical, Schumacher and Poser criteria. They maintain the requirement of dissemination of typical disease in time and space, by clinical, paraclinical, CSF and new MRI criteria. As with previous criteria, the footnotes are key to avoiding incorrect definite diagnoses of MS in situations that could be explained by,

for example, one multifocal bout of demyelination. Careful attention must also be paid to the conjunctions 'and' or 'or' in the various data combinations being used to support an MS diagnosis. As always, the caveat that no better explanation or diagnosis can be found applies, with MRI certainly helping to rule out or differentiate among the differential diagnoses of MS.

The McDonald criteria simplify the Poser criteria by including only visual evoked response tests and not brainstem or somatosensory modalities as paraclinical evidence to support an MS diagnosis. This reflects the concern that the latter two modalities are less sensitive at picking up demyelinating lesions and the fact that visual pathway involvement is so typical in MS. They do include CSF criteria in the Poser criteria, though eliminate use the qualifier 'laboratory supported'. A concern is that CSF oligoclonal IgG bands are not being detected as sensitively in general laboratory medicine practice as the reported 90–95% positive in earlier research studies.

The major focus of the McDonald criteria was to incorporate, in a rigorous fashion, the growing use of MRI to make MS diagnoses. MS lesions are typically periventricular and corpus callosum, but also occur in the centrum semiovale, internal capsule, cerebellum and cerebellar pathways. These lesions are best seen on T2 and proton density sequences and more chronic and sclerotic lesions appear as so-called 'black holes' on T1 sequences. Ovoid lesions that are perpendicular to the ventricles and corpus callosum on T2 sequences, are fairly specific to MS. The McDonald criteria specifically incorporate parameters of lesion location and numbers for typical geographic dissemination in MS in TABLE 3 [24,25]. Gadolinium contrast is another commonly used parameter to both increase the sensitivity of detecting MS lesions and to assess lesion activity [21,22]. An MS lesion typically enhances with gadolinium for approximately 1 month and then the enhancement resolves, leaving a smaller area of signal abnormality on T2 sequences. A single MRI scan of the brain demonstrating multifocal lesions, can be used to demonstrate geographic dissemination to help with an MS diagnosis, but cannot be used to provide evidence for temporal dissociation. Even if some lesions enhance with gadolinium and others do not, this does not prove temporal dissemination and can be consistent with an acute demyelinating event, such as ADEM. TABLE 4 is crucial, by emphasizing the additional need to see evolution of new typical T2 or gadolinium-enhancing MRI lesions over specific time-frames.

Table 3. MRI criteria to support MS Diagnosis – brain abnormality (from [23,24,25]).

Three out of four of the following:
One gadolinium-enhancing lesion or nine T2-hyperintense lesions if there is no gadolinium enhancing lesion
At least one infratentorial lesion
At least one juxtacortical lesion
At least three periventricular lesions

Table 4. Magnetic resonance imaging criteria to support MS Diagnosis: dissemination of lesions in time (from [23]).

If a first scan occurs 3 months or more after the onset of the clinical event, the presence of a gadolinium-enhancing lesion is sufficient to demonstrate dissemination in time, provided that it is not at the site implicated in the original clinical event. If there is no enhancing lesion at this time, a follow-up scan is required. The timing of this follow-up scan is not crucial, but 3 months is recommended. A new T2- or gadolinium-enhancing lesions at this time then fulfills the criterion for dissemination in time.
If the first scan is performed less than 3 months after the onset of the clinical event, a second scan carried out 3 months or more after the clinical event showing a new gadolinium-enhancing lesion provides sufficient evidence for dissemination in time. However, if no enhancing lesion is seen at this second scan, a further scan not less than 3 months after the first scan that shows a new T2 lesion or an enhancing lesion will suffice.

Though MRI scans of the brain are a very sensitive way of detecting MS demyelinating lesions and assessing disease activity, it must be kept in mind that MRI lesions in the brain are very nonspecific and a diagnosis of MS should not be made on MRI alone. It is also very unusual and raises concern about diagnosis, if a person with suspected MS has a consistently normal or statically abnormal MRI over a significant period of time. If an MS diagnosis is secure by other means, a normal or mildly abnormal MRI represents a good prognostic feature. On the other hand, higher disease burden at the time of a first demyelinating event increases the risk of subsequent conversion to clinically definite MS and higher disease burden at the time of an MS diagnosis is an indicator of worse clinical prognosis [26–28].

The McDonald criteria are already controversial. One concern is how much of the MRI criteria is truly evidence-based and other MRI-based criteria are being proposed. For example, a single typical MRI lesion in the spinal cord may carry more import than several nonspecific lesions in the brain, but this is not accounted for in the criteria. Another concern that may create confusion for patients and physicians is the elimination of the category of 'probable' MS, so that a patient can only be diagnosed with definite MS, possible MS, or 'not MS'. I still find the 'probable' qualifier useful in situations where there are acute multifocal presentations with variable gadolinium-enhancing quality to MRI brain lesions, or where there is a monosymptomatic demyelinating presentation with at least two typical white matter lesions of 3 mm or greater in size, which is known to place the patient at high risk of going on to have another temporally dissociated attack to then make the MS diagnosis. The distinction between the qualifiers 'possible' or 'probable' may not make a difference therapeutically, however. In monosymptomatic presentations with these MRI abnormalities, there is now evidence that immunotherapy should be initiated to decrease the frequency and delay the timing at which the next and MS-defining attack of demyelination occurs. Another criticism of these criteria is that they have

Table 5. McDonald criteria for MS diagnosis (from [23]).

Clinical presentation	Additional data needed for MS diagnosis
Two or more attacks; objective clinical evidence of two or more lesions	None[a]
Two or more attacks; objective clinical evidence of one lesion	Dissemination in space, demonstrated by MRI[b] or Two or more MRI-detected lesions consistent with MS plus positive CSF[c] or Await further clinical attack implicating a different site
One attack; objective clinical evidence of two or more lesions	Dissemination in time, demonstrated by MRI[d] or Second clinical attack
One attack; objective clinical evidence of one lesion (mono-symptomatic presentation; clinically isolated syndrome)	Dissemination in space, demonstrated by MRI[b] or Two or more MRI-detected lesions consistent with MS plus positive CSF[c] and Dissemination in time, demonstrated by MRI[d] or Second clinical attack
Insidious neurological progression suggestive of MS	Positive CSF[c] and Dissemination in space, demonstrated by: • Nine or more T2 lesions in brain • Two or more lesions in spinal cord • 4–8 brain plus 1 spinal cord lesions or abnormal VEP[e] associated with 4–8 brain lesions, or with fewer than four brain lesions plus one spinal cord lesion demonstrated by MRI and Dissemination in time, demonstrated by MRI[d] or Continued progression for 1 year

If criteria indicated are fulfilled, the diagnosis is multiple sclerosis (MS); if the criteria are not completely met, the diagnosis is 'possible MS'; if the criteria are fully explored and not met, the diagnosis is 'not MS'.

[a]No additional tests are required; however, if tests MRI and CSF tests are undertaken and are negative, extreme caution should be taken before making a diagnosis of MS. Alternative diagnoses must be considered. There must be no better explanation for the clinical picture.

[b]MRI demonstration of space dissemination must fulfill the criteria from Barkhof *et al.* [24] and Tintoré *et al.* [25] (TABLE 3).

[c]Positive CSF determined by oligoclonal bands detected by established methods (preferably isoelectric focusing) different from any such bands in serum or by a raised IgG index.

[d]MRI demonstration of time dissemination must fulfill the criteria listed in TABLE 4.

[e]Abnormal visual evoked potential of the type seen in MS (delay with a well-preserved wave form).

introduced prognostic aspects into the diagnostic process, though in the future this may be embraced as an advantage. Even as these newest criteria try to anticipate, incorporate and standardize all possible clinical nuances, there are whole articles still trying to define PPMS [28].

As MRI parameters and repeated MRI scans are increasingly used to help in the MS diagnostic process, there are unresolved issues. If a second brain MRI does not reveal dissemination of disease per TABLE 4 to make an MS diagnosis, there is no further evidence-based recommendation about how often to repeat MRI and at what interval and with what clinical indications. In addition, as we use comparison MRI in clinical practice and not just in the tightly controlled clinical research settings from which the newer MRI criteria were derived, there is a need to standardize the field strength, pulse sequences, slice thickness, gadolinium dose and landmarks, so that comparisons can validly be made. Scans carried out on different machines with different protocols are often not reliable at providing the precise information we are now asking of MRI. Newer scans carried out with better analytical software and on higher field strength magnets may demonstrate more lesions which are not true new disease activity, but just more sensitive detection. MRI recorded on radiographic hard-copy film may not be developed identically each time and may be hard to compare with newer MRI for which hard copies may no longer be printed and only electronic images available. Neuroradiologists are not used to reading out 'spot counts' and may report comparisons in broad brush strokes if at all, which again does not provide the precise information that our decision-making increasingly requires.

Differential diagnosis of MS

Whenever a diagnosis of MS is proposed, by whichever of the previously discussed criteria, there is always the caveat that no other disease process provides a better diagnosis (TABLE 6). Certain diagnoses should always be ruled out at least once in the early evaluation for MS and later if atypical attacks or unusual clinical or MRI findings occur. Possible MS cases should be screened for collagen vascular disease, looking for the unusual systemic lupus erythematosus case with a CNS presentation, or Sjogren's syndrome. Such testing includes sedimentation rate, antinuclear antibody, rheumatoid factor and rapid plasma reagin (RPR). Any positive test should be followed up with testing, such as: Sjogren's syndrome antigens-A and B, antidouble-stranded DNA, SCL-70, antiRNP, antisingle-stranded DNA. It must be kept in mind that typical MS patients have a low titer false-positive antinuclear antibody in about 25% of cases and other testing will be negative. If there is evidence for central and peripheral demyelination, or early or prominent occurrence of seizures, screening for Wegener's granulomatosus with antineutrophil cytoplasmic antibody (ANCA) and CNS vasculitis in isolation or with systemic manifestations with cerebral arteriography (MR angiography is not likely sensitive enough yet to rule out vasculitis of the small and medium size vessels typically affected by CNS vasculitis). Presence of oral and genital ulcers, especially in individuals of Middle Eastern descent, suggests

Table 6. Differential diagnosis of MS (partially from [1,4,6,30]).

1.	Acute isolated demyelinating event: optic neuritis, transverse myelitis, Devic's syndrome, other
2.	Acute multifocal demyelinating event: acute or postinfectious encephalomyelitis, Marburg's
3.	Vasculitis: isolated CNS, system lupus erythematosus, Wegner's granulomatosis, polyarteritis nodosa
4.	Nutritional/endocrine: hypothyroid, subacute combined degeneration
5.	Subacute or chronic infections: HTLV-I, toxoplasmosis, tuberculosis, neurosyphilis, Lyme, progressive multifocal leukoencephalopathy, HIV, *Herpes zoster*
6.	Neurosarcoidosis
7.	Sjogren's syndrome
8.	Bechet's syndrome
9.	Multiple strokes: thrombotic/lacunar, multiple embolic, subacute bacterial endocarditis, hypercoaguable state, anticardiolipin antibody syndrome, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)
10.	Multiple metastases, CNS lymphoma, paraneoplastic syndrome
11.	Spinal cord compressive lesion: tumor, disc, spondylosis, Arnold-Chiari malformation
12.	Complicated migraine
13.	Dysmyelinating condition, carrier of gene for: adrenoleukodystrophy (adreno myeloneuropathy), Tay Sachs disease, metachromatic leukodystrophy
14.	Hereditary ataxias and paraplegias, Leber's optic atrophy

Bechet's syndrome. Another entity that can present with multifocal CNS and PNS involvement is sarcoidosis, which can be initially screened for by serum and/or CSF angiotensin-converting enzyme (ACE) level and pursued depending upon clinical suspicion with chest x-ray, pulmonary function tests with diffusing capacity, serum calcium and gallium scanning. ACE levels can be falsely elevated in otherwise typical MS. Other diseases that can cause multifocal CNS involvements include thyroid dysfunction and vitamin B12 deficiency. Individuals with vascular disease risk-factors should be evaluated with: neuroimaging (acutely with CT if thrombolysis may be appropriate, otherwise with MRI), MR angiography and or ultrasound of the carotid arteries, echocardiography looking for sources of emboli and lipid profile. Presence of fever suggests infective endocarditis with septic emboli. Association of headache suggests complicated migraine or cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) Young onset of vascular events warrants investigation for hypercoagulable state, including: PT/PTT, protein C and S levels, antithrombin III level, Factor V Leiden, homocysteine level and screening for anticardiolipin antibodies. CSF analysis for the typical IgG abnormalities of MS is particularly useful at distinguishing multifocal demyelination from multifocal subcortical vascular disease. If there is a prominent family history suggestive of MS, early prominent cognitive involvement or dementia, seizures, or evidence of CNS and PNS demyelination, it can be worth screening for carriers of the genes for adrenoleukodystrophy (very long chain fatty acid level) who can develop adrenomyeloneeuropathy, Tay Sachs disease (hexosaminidase A level), or metachromatic leukodystrophy (arylsulfatase A level). In a case of suspected PPMS, it is crucial to rule out a compressive spinal cord lesion – intrinsic or extrinsic – and also a Chiari malformation or cerebellar degeneration, using spinal MRI or myelography if spinal MRI cannot be accomplished.

Early in the work-up for suspected MS, serological evaluation can rule out subacute or chronic infections with HIV, Lyme disease, human T-cell lymphocytic virus (HTLV-I), or syphilis and CSF evaluation can reveal atypical protein or white blood cell elevations or cultures that indicate subacute or chronic infections, such as Lyme, tuberculosis (TB), or fungus. MS patients can also have false positive Lyme titers.

Expert opinion

Major questions still to be answered, are: whether the use of 'splitting' terms like RRMS, SPMS, PPMS and PRMS, are meaningful clinically, pathologically, or in predicting responses to immunotherapies or to newer neuroprotective therapies in development; whether newer more meaningful 'splits' can be made based on pathological, MRI, CSF, or peripheral blood criteria; or whether MS will in the short or longer term be 'lumped' as one disease with therapies available that are started not dependent on more specific subcategorization.

Five-year view

Though creating some initial controversy, I suspect that the McDonald criteria will become second nature to neurologists engaged in the MS diagnostic process, as did the Poser criteria before them. There will be workshops and meetings and publications that help us all get familiar with and comfortable using the new criteria. Loop-holes and inconsistencies will be found and corrected or clarified, but I do not think there will be a need for a whole new set of criteria over the next 5 years.

The MS diagnostic process will likely begin to incorporate techniques such as magnetic transference resonance (MTR), diffusion sequences, or MR spectroscopy, all of which may increase the sensitivity of detected MS lesions and may begin to allow better differentiation of different components of the MS disease process, such as: inflammation, edema, blood–brain barrier breakdown and axonal degeneration. Software programs to measure and monitor atrophy, with diagnostic and prognostic implications, are likely to be available and clinically useful soon. Quick (and therefore cheap) MRI protocols will likely become available, some of which may give one or a

few reproducible measurements that will be shown to have diagnostic and prognostic implications and can be used to monitor the MS disease process 'an MS sed rate'. As part of the diagnostic process, parameters, such as cytokine profiles, HLA types and apolipoprotein E haplotypes will be measured to provide additional confirmatory evidence of diagnosis, but more importantly, will have prognostic implications.

Key issues

- There must be objective evidence of dissemination of typical demyelinating disease in both time and space to be sure of an multiple sclerosis (MS) diagnosis and to make sure the diagnosis is not an acute mono- or multifocal demyelination that may never reoccur. No single event of demyelination, no matter how multifocal by clinical, MRI, or evoked potential testing parameters, can result in a definite MS diagnosis.
- There must be a second temporally dissociated objectively proven event of demyelination to make an MS diagnosis and that event could be a previous historical event, or a prospective future event.
- A patient with a clinical event of mono- or multifocal demyelination who has a previous suggestive historical event of demyelination, can be diagnosed with definite MS if the previous event can be objectively collaborated by a neurological examination sign, or by an evoked potential abnormality of the relevant pathway, or by cerebrospinal fluid (CSF) showing oligoclonal immunoglobulin (Ig)G bands or elevated IgG index, or by a current MRI scan fulfilling the criteria for dissemination in space. If the patient with a current episode of mono- or multifocal demyelination does not have a previous history suggestive of demyelination, or if the above ways to objectively support a historical event of demyelination are not helpful, then a definite diagnosis of MS cannot be made. The McDonald criteria only allow use of 'possible' MS in such situations, but Poser criteria also allow use of 'probable' MS, which may be more accurate and helpful to patients in some situations.
- To prospectively diagnose definite MS in cases of a mono- or multifocal episode of demyelination with no prior history suggestive of demyelination, or where the previously discussed ways to objectively support a historical event of demyelination are not successful, one must wait for a new event consistent with typical demyelination to occur. The classic and gold standard new event, at greater than a month after the first one, would be a new geographically dissociated symptomatic attack of demyelination, with objective abnormality on neurologic exam. Evolution of a subclinical definitely new neurologic exam finding would also suffice, albeit at a low sensitivity. Similarly, a new clinical event, typically sensory or visual, without objective new findings on neurological examination, could be supported objectively as being on the basis of CNS demyelination if the relevant evoked potential is abnormal. If initially normal CSF evolves to demonstrate abnormal IgG index or oligoclonal IgG bands, with or without suspicious clinical episodes, this would allow a definite MS diagnosis. Patients are unlikely to allow a second lumbar puncture, however, so it can be better to wait to carry out lumbar puncture until further suspicious clinical activity has occurred and CSF is more likely to be abnormal and helpful, unless early differential diagnosis depends on acute examination of the CSF.
- Prospectively repeating the MRI brain is the fastest and most sensitive way to definitely diagnose MS, in the era when quick diagnosis is desired to eliminate uncertainty for the patient and to recommend initiation of maintenance immunotherapy to decrease future disease activity as best we can. A repeat MRI brain comparable in technique to the first one, that shows a definitely new gadolinium-enhancing lesion at a 3 month interval, or a new T2 signal lesion at a 6 month interval, provides a definite MS diagnosis.
- In cases of an initial isolated demyelinating event, such as optic neuritis, transverse myelitis, or another brainstem event, where the diagnosis must be left as 'possible' or 'probable' MS, the lack of a definite diagnosis of MS does not mean that therapeutic recommendations cannot be made. In such cases, if MRI brain demonstrates at least two lesions of greater than or equal to 3 mm in size that do not explain the clinical syndrome, there is class I evidence that initiating interferon-β1a can delay or prevent the next MS-defining attack from occurring.

References

Papers of special note have been highlighted as:
- • of interest
- •• of considerable interest

1 Noseworthy JH, Lucchinetti C, Rodriguez M *et al.* Multiple sclerosis. *N. Engl. J. Med.* 343(13), 938–952 (2000).

2 Lubin FD, Reingold SC. Defining the clinical course of multiple sclerosis: Results of an international survey. *Neurology* 46, 907–911 (1996).

3 Weinshenker BG, Bass B, Rice GPA *et al.* The natural history of multiple sclerosis: A geographic based study. I. Clinical course and disability. *Brain* 112, 133–146 (1989).

4 Matthews WB, Acheson ED, Batchelor JR *et al. McAlpine's Multiple Sclerosis.* Churchill Livingstone, Edinburgh, London, Melbourne and New York 146–209 (1985).

5 Schumacher FA, Beebe G. Kibler RF *et al.* Problems of experimental trials of therapy in multiple sclerosis: Report by the panel on the evaluation of experimental trials of therapy in multiple sclerosis. *Ann. NY Acad. Sci.* 122, 552–568 (1965).

•• **Schumacher's seminal attempt to standardize the clinical diagnosis of MS.**

6 Paty DW, Ebers GC. *Multiple sclerosis.* FA Davis Company, Philadelphia, PA, USA 48–134 (1998).

7 Beck R, for the Optic Neuritis Study Group. The 5-year risk of MS after optic neuritis. Experience of the Optic Neuritis

Treatment Trial. *Neurology* 49, 1404–1413 (1997).

8 Miller DH, Ormerod IEC, Rudge P, Kendall RE, Moseley IF, McDonald WI. The early risk of multiple sclerosis following isolated acute syndromes of the brainstem and spinal cord. *Ann. Neurology* 26, 635–639 (1989).

9 Kesselring J, Miller DH, Robb SA *et al.* Acute disseminated encephalomyelitis: MRI findings and the distinction from multiple sclerosis. *Brain* 113, 291–302 (1990).

10 Schwartz S, Knauth M, Wildemann B *et al.* Acute disseminated encephalomyelitis. A follow-up study of 40 adult patients. *Neurology* 56, 1313–1318 (2001).

11 Weinshenker BG, Bass B, Rice GPA *et al.* The natural history of multiple sclerosis: A geographically based study. II. Predictive value of the early clinical course. *Brain* 112, 1419–1428 (1989).

12 Confavreux C, Vukusic S, Moreau T *et al.* Relapses and progression of disability in multiple sclerosis. *N. Engl. J. Med.* 343(20), 1430–1485 (2000).

• Newest clinical epidemiologic characterization of MS.

13 Herndon RM, Brooks B. Misdiagnosis of multiple sclerosis. *Semin. Neurology* 5(2), 94–98 (1985).

14 Poser CM, Paty DW, Scheinberg L *et al.* New diagnostic criteria for multiple sclerosis: Guidelines for research protocols. *Ann. Neurol.* 13(3), 227–231 (1983).

•• Poser criteria which added evoked potentials and CSF parameters to the MS standardized diagnostic criteria.

15 Gronseth GS, Ashman EJ. Practice parameter: The usefulness of evoked potentials in identifying clinically silent lesions in patients with suspected multiple sclerosis (an evidence-based review). *Neurology* 54, 1720–1725 (2000).

16 Andersson M, Alvarez-Cermeno J, Bernardi G *et al.* Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. *J. Neurol. Neurosurg. Psych.* 57, 897–902 (1994).

• Excellent review of how CSF fits into the MS diagnostic process.

17 Paolino E, Fainardi E, Ruppi P *et al.* A prospective study on the predictive value of CSF oligoclonal bands and MRI in acute isolated neurological syndromes for subsequent progression to multiple sclerosis. *J. Neurol. Neurosurg. Psychiatr.* 60, 572–575 (1996).

18 Lee KH, Hashimoto SA, Hooge JP *et al.* Magnetic resonance imaging of the head in the diagnosis of multiple sclerosis: A prospective 2-year follow-up with comparison of clinical evaluation, evoked potentials, oligoclonal banding and CT. *Neurology* 41, 657–660 (1991).

19 Paty DW, Asbury AK, Herndon RM *et al.* Use of magnetic resonance imaging in the diagnosis of multiple sclerosis: Policy statement. *Neurology* 36, 1575 (1986).

20 Paty DW, McFarlin DE, McDonald WI. Magnetic resonance imaging and laboratory aids in the diagnosis of multiple sclerosis. *Ann. Neurol.* 29(1), 3–5 (1991).

21 Fazekas F, Barkhof F, Filippi M *et al.* The contribution of magnetic resonance imaging to the diagnosis of multiple sclerosis. *Neurology* 53, 448–456 (1999).

22 Goodkin DE, Rudick RA, Ross JS. The use of brain magnetic resonance imaging in multiple sclerosis. *Arch. Neurol.* 51, 505–516 (1994).

23 McDonald WI, Compston A, Edan G *et al.* Recommended diagnostic criteria for multiple sclerosis: Guidelines from the international panel on the diagnosis of multiple sclerosis. *Ann. Neurol.* 50(1), 121–127 (2001).

•• McDonald criteria incorporating MRI parameters into the MS diagnostic process.

24 Barkhof F, Filippi M, Miller DH *et al.* Comparison of MR imaging criteria at first presentation to predict conversion to clinically definite multiple sclerosis. *Brain* 120, 2059–2069 (1977).

• Best characterization of MRI parameters useful in the MS diagnostic process.

25 Tintore M, Rovira A, Martinez M *et al.* Isolated demyelinating syndromes: comparison of different MR imaging criteria to predict conversion to clinically definite multiple sclerosis. *Am. J. Neuroradiol.* 21, 702–706 (2000).

26 Sailer M, O'Riordan JI, Thompson AJ *et al.* Quantitative MRI in patients with clinically isolated syndromes suggestive of demyelination. *Neurology* 53, 599–605 (1999).

27 Filippi M, Horsfield MA, Morrissey SP *et al.* Quantitative brain MRI lesion load predicts the course of clinically isolated syndromes suggestive of multiple sclerosis. *Neurology* 44, 635–641 (1994).

28 Khoury SJ, Guttman CRG, Orav EJ *et al.* Longitudinal MRI in multiple sclerosis: Correlation between disability and lesion burden. *Neurology* 44, 2120–2124 (1994).

29 Thompson AJ, Montalban X, Barkhof F *et al.* Diagnostic criteria for primary progressive multiple sclerosis: A position paper. *Ann. Neurol.* 47, 831–835 (2000).

30 Rudick RA, Ransohoff RM, Herndon RM. Multiple Sclerosis and other myelin disorders. Clinical Neurology Joynt RJ, Griggs RC (Ed.) *Neurology* 3(33), 1–106 (1998).

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SPECIAL REPORT

Recommended Diagnostic Criteria for Multiple Sclerosis: Guidelines from the International Panel on the Diagnosis of Multiple Sclerosis

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The International Panel on MS Diagnosis presents revised diagnostic criteria for multiple sclerosis (MS). The focus remains on the objective demonstration of dissemination of lesions in both time and space. Magnetic resonance imaging is integrated with clinical and other paraclinical diagnostic methods. The revised criteria facilitate the diagnosis of MS in patients with a variety of presentations, including "monosymptomatic" disease suggestive of MS, disease with a typical relapsing-remitting course, and disease with insidious progression, without clear attacks and remissions. Previously used terms such as *"clinically definite"* and *"probable MS"* are no longer recommended. The *outcome of a diagnostic evaluation is* either MS, "possible MS" (for those at risk for MS, but for whom diagnostic evaluation is equivocal), or "not MS."

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Because no single clinical feature or diagnostic test is sufficient for the diagnosis of multiple sclerosis (MS), diagnostic criteria have included a combination of both clinical and paraclinical studies.[1,2] The last formal review of criteria for MS diagnosis occurred in 1982,[2] at which time degrees of diagnostic certainty were identified by categories ranging from clinically definite diagnosis to laboratory-supported definite MS, clinically probable MS, and laboratory-supported probable MS.

In July, 2000, the International Panel on the Diagnosis of MS was convened in London, United Kingdom, under the auspices of the U.S. National Multiple Sclerosis Society and the International Federation of MS Societies to reassess existing diagnostic criteria and to recommend, if necessary, appropriate changes. The Panel set out to create diagnostic criteria that could be used by the practicing physician and that could be adapted, as necessary, for clinical trials. The Panel also set out to integrate magnetic resonance imaging (MRI) into the overall diagnostic scheme because of its unique sensitivity to pathological change and to include a scheme for the diagnosis of primary progressive disease[3]—that characterized by the absence of relapses or remissions from onset—because neither had been sufficiently defined or integrated into existing diagnostic criteria for MS. The Panel also sought to clarify certain definitions currently used in the diagnosis of MS and, when possible, to simplify the diagnostic classification and descriptions. While refining the diagnostic criteria to reflect improved understanding of the disease and new technologies, the Panel wished to retain as many as possible of the useful features of existing criteria. Among general outcomes of the discussion, the Panel concluded the following.

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- Obtaining objective evidence of dissemination in time and space of lesions typical of MS is essential in making a secure diagnosis, as is the exclusion of other, better explanations for the clinical features.
- Clinical evidence depends primarily on objectively determined clinical signs. Historical accounts of symptoms may lead to a suspicion of the disease but cannot be sufficient on their own for a diagnosis of MS. A diagnosis of MS on purely clinical evidence remains possible if there is objective evidence of lesions separated in time and space.
- Radiological and laboratory investigations, including MRI, analysis of cerebrospinal fluid (CSF), and visual evoked potentials (VEP), can add to a clinical diagnosis and may be essential in making a diagnosis when clinical presentation alone does not allow a diagnosis to be made. These tests provide different types of information, and their value depends on the context in which the diagnosis is being made. Each has limitations of sensitivity and specificity. Imaging is viewed as the most sensitive and specific of these in making an MS diagnosis. Because CSF adds a different kind of information—about inflammation and immunological disturbance—it may be useful in situations when the clinical picture is unusual or the imaging criteria for diagnosis are not fulfilled. VEP may provide additional support, particularly in situations in which MRI abnormalities are few (eg, in patients with primary progressive MS with progressive myelopathy) or when MRI abnormalities have lesser specificity (eg, in older individuals with risk factors for microvascular ischemic disease or in individuals with abnormal radiological findings that do not satisfy the MRI specificity criteria for diagnosis). Other types of evoked potential analysis were viewed as contributing little to the diagnosis of MS.[4]
- Following a diagnostic evaluation, an individual is usually classified either as having MS or as not having MS. A patient with appropriate clinical presentation who has not yet been evaluated, or whose evaluation meets some but not all of the necessary criteria, is considered to have "possible MS." Subcategories that define the types of studies used in the diagnostic workup ("clinically definite," "laboratory supported," etc.) are unnecessary.

Definitions

The Panel reviewed definitions used in previous diagnostic criteria to clarify terms for future diagnostic purposes.

What Constitutes an "Attack"?

An "attack" (exacerbation, relapse) refers to an episode of neurological disturbance of the kind seen in MS, when clinicopathological studies have established that the causative lesions are inflammatory and demyelinating in nature. Although there was some divergence of opinion, the group agreed that, for general diagnostic purposes, an attack, defined either by subjective report or by objective observation, should last for at least 24 hours.[2] This assumes that there is expert clinical assessment that the event is not a pseudoattack, such as might be caused by a change in core body temperature[5] or infection. Whereas suspicion of an attack may be provided by subjective historical reports from the patient, objective clinical findings of a lesion are required to make a diagnosis of MS. Single paroxysmal episodes (eg, a tonic spasm) do not constitute a relapse, but multiple episodes occurring over not less than 24 hours do.

How Is the Time Between Attacks Measured?

In defining what constitutes separate attacks, for the purposes of documenting separation in time of such events, it was agreed that 30 days should separate the onset of the first event from the onset of a second event. This interpretation has the advantage of being less ambiguous than considering the interval from beginning of recovery from the first event to initiation of the second event, as suggested in the definition of the "Poser Committee."[2]

How Is "Abnormality" in Paraclinical Tests Determined?

MRI. Lesions in the brain detected by MRI can provide evidence of dissemination of lesions in both time and space. It was agreed that stringent criteria for MRI abnormality should be followed in making an MS diagnosis. From among those that have been proposed, the Panel preferred those derived from the studies of Barkhof et al[6] and Tintoré et al,[7] which require evidence of at least three of four of the following: 1) one gadolinium-enhancing lesion or nine T2 hyperintense lesions if gadolinium-enhancing lesions are not present; 2) at least one infratentorial lesion; 3) at least one juxtacortical lesion (ie, involving the subcortical u-fibers); 4) at least three periventricular lesions (see Table 1). Lesions will ordinarily be larger than 3 mm in cross section. These criteria provide an acceptable degree of sensitivity while providing greater specificity and accuracy[7] than the MRI criteria proposed by Fazekas et al[8,9] and Paty et al.[10]

The assessment of dissemination in time is discussed below in relation to each particular mode of clinical presentation (see Table 2). The criteria derived from Barkhof et al[6] do nor deal with lesions detected in the

Table 1. Magnetic Resonance Imaging Criteria for Brain Abnormality

Three of four of the following
1. One gadolinium-enhancing lesion or nine T2-hyperintense lesions if there is no gadolinium enhancing lesion
2. At least one infratentorial lesion
3. At least one juxtacortical lesion
4. At least three periventricular lesions

Note: One spinal cord lesion can be substituted for one brain lesion. Data from Barkhof et al[6] and Tintoré et al.[7]

spinal cord. Prospective data are currently insufficient to define more precisely the role of spinal cord lesions in diagnosis. However, the characteristics and distribution of spinal cord lesions in MS are well-described, as is their absence in healthy controls, even among older adults.[11] There should be little or no swelling of the cord, although exceptions occur, and such spinal lesions should be unequivocally hyperintense on T2-weighted images, be at least 3 mm but under two vertebral segments in length, and occupy only part of the cross section of the cord.[12] Accordingly, spinal cord lesions detected by MRI might, in some situations (such as in clinically isolated syndromes[13] or when disease is progressive from onset[3]), supplement incomplete information from brain MRI scans. Whereas it is possible that, in the absence of brain lesions, two or more spinal cord lesions clearly separated in time and/or space could satisfy criteria, prospective data in this regard are still awaited. It is expected that with further research the necessary information on sensitivity and specificity of spinal cord images for MS diagnosis will be available.

CSF ANALYSIS. Abnormality on CSF analysis can provide supportive evidence of the immune and inflammatory nature of lesion(s), which may be helpful when imaging criteria fall short, when they lack specificity (as in the older patient), or when the clinical presentation is atypical. CSF analysis cannot provide information about dissemination of lesions or events in time or space.

For the purpose of diagnosing MS, CSF abnormality is defined (preferably using isoelectric focusing) by the presence of oligoclonal IgG bands different from any such bands in serum and/or the presence of an elevated IgG index.[14,15] Lymphocytic pleocytosis should be less than 50/mm^3. It is recognized that the quality of CSF analysis is not uniform among laboratories, regions, or countries. It is the practitioner's obligation, when including results of such analyses, to ensure that they are being done in the most reproducible fashion, with state-of-the-art technology. Failure to do so might result in unreliable measurement and incorrect diagnosis.

VEP. Abnormal VEP, typical of MS (delayed but with well-preserved wave form[16]), can be used to supplement information provided by a clinical examination[4] to provide objective evidence of a second lesion provided that the only clinically expressed lesion did not affect the visual pathways. As with MRI and CSF analysis, correct interpretation is essential.

The Diagnostic Scheme

Table 3 indicates the steps that should be undertaken in making a diagnosis of MS. In this scheme, the mode of clinical presentation is indicated in the left column. The data needed to make an MS diagnosis are indicated, for each presentation, in the right column. Failure to satisfy the criteria for an MS diagnosis will result in either a "possible MS" diagnosis, pending further analysis, or classification as "not MS." The order in the table of "clinical presentation" is deliberate; the Panel believes that a diagnosis is simplest in the case of "two attacks, clinical evidence of two or more lesions" and becomes increasingly difficult through "insidious neurological progression suggestive of MS." The additional criteria needed to make a diagnosis of MS, therefore, become more stringent as the clinical evidence upon presentation becomes weaker. As is made clear below, follow-up with additional clinical assessments, laboratory investigation, and in particular MRI is important when a diagnosis cannot be made on clinical criteria alone at first presentation.

Two or More Attacks, Objective Clinical Evidence of Two or More Lesions

Two clear attacks typical of MS, documented by objective evidence of two lesions separated in time and necessarily separated in space may be sufficient to make an MS diagnosis solely on clinical grounds. No additional tests may be needed. However, it would be expected that

Table 2. Magnetic Resonance Imaging Criteria for Dissemination of Lesions in Time

1. If a first scan occurs 3 months or more after the onset of the clinical event, the presence of a gadolinium-enhancing lesion is sufficient to demonstrate dissemination in time, provided that it is not at the site implicated in the original clinical event. If there is no enhancing lesion at this time, a follow-up scan is required. The timing of this follow-up scan is not crucial, but 3 months is recommended.[22] A new T2- or gadolinium-enhancing lesion at this time then fulfills the criterion for dissemination in time.
2. If the first scan is performed less than 3 months after the onset of the clinical event, a second scan done 3 months or more after the clinical event showing a new gadolinium-enhancing lesion provides sufficient evidence for dissemination in time. However, if no enhancing lesion is seen at this second scan, a further scan not less than 3 months after the first scan that shows a new T2 lesion or an enhancing lesion will suffice.

Table 3. Diagnostic Criteria

Clinical Presentation	Additional Data Needed for MS Diagnosis
Two or more attacks; objective clinical evidence of 2 or more lesions	None[a]
Two or more attacks; objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by MRI[b] *or* Two or more MRI-detected lesions consistent with MS plus positive CSF[c] *or* Await further clinical attack implicating a different site
One attack; objective clinical evidence of 2 or more lesions	Dissemination in time, demonstrated by MRI[d] *or* Second clinical attack
One attack; objective clinical evidence of 1 lesion (monosymptomatic presentation; clinically isolated syndrome)	Dissemination in space, demonstrated by MRI[b] *or* Two or more MRI-detected lesions consistent with MS plus positive CSF[c] *and* Dissemination in time, demonstrated by MRI[d] *or* Second clinical attack
Insidious neurological progression suggestive of MS	Positive CSF[c] *and* Dissemination in space, demonstrated by 1) Nine or more T2 lesions in brain *or* 2) 2 or more lesions in spinal cord, *or* 3) 4–8 brain plus 1 spinal cord lesion *or* abnormal VEP[e] associated with 4–8 brain lesions, or with fewer than 4 brain lesions plus 1 spinal cord lesion demonstrated by MRI *and* Dissemination in time, demonstrated by MRI[d] *or* Continued progression for 1 year

If criteria indicated are fulfilled, the diagnosis is multiple sclerosis (MS); if the criteria are not completely met, the diagnosis is "possible MS"; if the criteria are fully explored and not met, the diagnosis is "not MS."

[a]No additional tests are required; however, if tests [magnetic resonance imaging (MRI), cerebral spinal fluid (CSF)] are undertaken and are *negative*, extreme caution should be taken before making a diagnosis of MS. Alternative diagnoses must be considered. There must be no better explanation for the clinical picture.

[b]MRI demonstration of space dissemination must fulfill the criteria derived from Barkhof et al[6] and Tintoré et al[7] (see Table 1).

[c]Positive CSF determined by oligoclonal bands detected by established methods (preferably isoelectric focusing) different from any such bands in serum or by a raised IgG index.[14,15]

[d]MRI demonstration of time dissemination must fulfill the criteria listed in Table 2.

[e]Abnormal visual evoked potential of the type seen in MS (delay with a well-preserved wave form).[16]

one or more such tests—MRI, CSF, or VEP—would be abnormal were they done. If these tests are undertaken and are *not* abnormal in a manner typical of MS, extreme caution must be taken in making a diagnosis of MS. It must be stressed that there should be no better explanation than MS for the clinical picture.

Two or More Attacks, Objective Clinical Evidence of One Lesion

To make a diagnosis of MS, objective evidence of a second lesion is required to demonstrate dissemination in space. This can be provided by an MRI scan of the brain fulfilling criteria derived from Barkhof et al[6] and Tintoré et al[7] (see Table 1). A spinal cord lesion can be substituted for one of the brain lesions. Alternatively, should MRI data fall short of these requirements, the presence of at least two brain lesions or one brain and one spinal cord lesion consistent with MS, coupled with abnormal CSF analysis (to avoid misdiagnosing nonspecific vascular lesions as inflammatory), can be used to document dissemination in space. Alternatively, if MRI is not performed, the occurrence of a

further clinical attack implicating a different site will fulfill criteria for dissemination in space.

One Attack, Objective Clinical Evidence of Two or More Lesions

To make a diagnosis of MS, dissemination in time must be demonstrated. This can be done by MRI, although careful consideration must be given to the timing of the clinical event and subsequent scans (see Table 2). There must be a minimum of 3 months between the clinical event and evidence for a new lesion. (This interval is arbitrary, but it reduces the risk of misdiagnosing MS in cases of acute disseminated encephalomyelitis with a stuttering onset.[17]) Alternatively, if MRI tests are not performed, the occurrence of a second clinical attack is necessary to fulfill criteria for dissemination in time.

One Attack, Objective Clinical Evidence of One Lesion

To make a diagnosis of MS, dissemination of lesions both in space and in time will have to be demonstrated. The typical situation is the patient presenting solely with a clinically isolated syndrome suggestive of MS (so-called monosymptomatic presentation). A diagnosis of MS then requires 1) evidence of dissemination in space through detection of lesions using MRI as described above (see also Table 1) or, lacking such solid evidence, at least two brain lesions plus positive CSF, *and* 2) evidence of dissemination in time demonstrated as for the patient presenting with one attack and clinical evidence of two lesions (see above and Tables 2, 3). In this situation as well, if MRI tests are not performed, the occurrence of a second clinical attack implicating a different site will fulfill criteria for dissemination in time and space.

Insidious Neurological Progression Suggestive of Multiple Sceloris

This is often a difficult presentation for a diagnosis of MS, in that typical relapses are absent and dissemination in time and in space of separate events may be difficult to determine. The Panel had particular difficulty in reaching a consensus on the criteria for diagnosis in this clinical group, because the amount of published follow-up data for this is much less than for other modes of clinical presentation. For this reason, the stringent criteria proposed in a recent position paper[3] serve as the basis for the proposed diagnostic criteria. The Panel recognizes that modifications may be appropriate as more information becomes available.

With this mode of clinical presentation, to make a secure diagnosis of MS, the majority of the Panel considered that an abnormal CSF finding with evidence of inflammation and immune abnormality is essential and that evidence is required of dissemination in space (using MRI or abnormal VEP) and time (using MRI or continued progression of disability for 1 year). When these criteria are fulfilled, the diagnosis is "primary progressive MS" (see Table 3).

No Better Explanation

The Panel emphasizes that, even if the clinical evidence and paraclinical studies are strongly indicative of MS, there must be *no better explanation* for the clinical and paraclinical abnormalities than MS for a secure diagnosis to be made.

Discussion

The diagnosis of MS has traditionally relied upon accumulation of information, clinical and paraclinical, that leads to a positive diagnosis and can help to eliminate alternative diagnoses. Among the key indicators is evidence that the disease is inflammatory, whether recurrent or progressive. The International Panel on the Diagnosis of MS reaffirms the need to demonstrate dissemination of clinical events and lesions in space and time, long-held criteria for MS diagnosis, and the diagnostic scheme presented is organized to emphasize this point. Requiring objective clinical evidence of attacks or progression (symptoms alone are not enough) is a renewed emphasis but one that the Panel believes is essential because of the implications of the diagnosis of MS for treatment.

The criteria presented in this report are intended for use by the practicing physician, and it is expected that in most cases these clinicians will have access to the technologies required for the diagnostic workup. However, the Panel recognizes that in some parts of the world access to advanced technologies such as MRI is limited; if so, and if no alternatives to imaging (such as analysis of CSF and VEP) are available, a diagnosis of "possible MS" will be made until the subsequent clinical course allows the criteria of at least two attacks and clinical evidence of at least two separate lesions to be fulfilled.

It is further recognized that the methods and sensitivity of paraclinical testing and analysis vary worldwide. The Panel's recommendations are predicated on the availability of highest quality, state-of-the-art technology related to imaging, CSF analysis, and evoked potential recording. For example, in the use of imaging to document dissemination of lesions in time, accurate repositioning and coregistration of scans may be necessary to determine whether some of the lesions appearing on a follow-up scan are new.[18] When a physician is not ensured of the quality and reproducibility of any paraclinical analyses, extreme care must be taken in using the results as evidence supporting a diagnosis of MS. It is hoped that these recommendations will encourage greater uniformity and reliability in the use of such technologies.

The Panel's recommendations represent a pragmatic approach to allow a diagnosis of MS in the most typical clinical presentations. It is important to note that the recommendations are based on data and experience available primarily from adults with typical features of MS and that these criteria would best apply to individuals between 10 and 59 years of age and in cases in which the clinical presentation is reasonably suggestive of MS. Special care must be taken in making a diagnosis of MS in those who are younger or older at presentation, those with a progressive onset, and those with unusual features or an "atypical" presentation, such as dementia, epilepsy, or aphasia. In such cases, additional evidence from CSF and VEP analysis may help in attaining security about a diagnosis of MS, even if these are not required for the more typical cases. For unusual cases, the importance of follow-up assessments cannot be overemphasized.

Several MS-like presentations and clinical syndromes present particular difficulties in considering a diagnosis of MS. A detailed discussion of differential diagnosis is beyond the scope of this paper, and the reader is referred to standard accounts of differential diagnosis.[19] Nevertheless, several conditions that may be confused with MS should be kept in mind in assessing a patient for an MS diagnosis. These include multifocal areas of cerebral ischemia or infarction in young adults from such illnesses as phospholipid antibody syndrome, acute disseminated lupus erythematosis, CADASIL, Takayasu's disease, meningovascular syphilis, or even carotid dissecrion. Various infections such as HTLV1 and Lyme disease can present striking similarities to MS. Cerebellar ataxia presenting as a result of a paraneoplastic disorder in young adults may be a problem, especially because elevated IgG often occurs in the CSF in this illness. Monophasic demyelinating diseases such as acute disseminated encephalomyelitis, postviral Devic's syndrome, and some cases of acute transverse myelitis present special difficulties in diagnosis; a diagnosis should not be made in these circumstances unless new symptoms and signs or imaging abnormalities appear more than three months after clinical onset. Some regard recurrent demyelinating diseases such as acute disseminated encephalomyelitis with a stuttering onset, neuromyelitis optica (Devic's syndrome[20]), and recurrent longitudinally extensive transverse myelitis as separate diseases, but others regard them as variants of MS. Genetic disorders of myelin, such as the leukodystrophies, should be considered in certain settings, particularly among children and teenagers.

Clinical trials for evaluating new therapeutic agents and other clinical experimental protocols may require different diagnosis-related inclusion and exclusion criteria than those provided in the present recommended basic steps. Given the wide variation in presentation of MS, there must be some flexibility in the application of the new diagnostic scheme. A secure diagnosis, however, should be based on the elements presented here. It must always be remembered that there should be no better explanation for the clinical and investigative data obtained.

Whereas it might be said that the only proved diagnosis of MS can be made upon autopsy,[21] or occasionally upon biopsy, where lesions typical of MS can be directly detected through standard histopathological techniques, MS is essentially a clinical problem and can be diagnosed using clinical and paraclinical criteria. Biopsy is a diagnostic technique that can confirm that a lesion is inflammatory and demyelinating (though it cannot on its own lead to a diagnosis of MS) and should *rarely* be undertaken. Interpretation by neuropathologists experienced in the demyelinating diseases is essential in avoiding misdiagnosis.

Imaging undertaken for other purposes occasionally uncovers "silent disease." When such silent cases are uncovered, some degree of monitoring may be desirable.

The International Panel on MS Diagnostic Criteria built upon diagnostic recommendations for MS that have served the community well for decades. Key points include a continued emphasis on dissemination of lesions in time and space and on the value of paraclinical testing, especially imaging, as a key part of the overall diagnostic workup. Specific imaging criteria are presented. However, the diagnosis of MS remains a partly subjective and partly objective process. The diagnosis is best made by an expert who is familiar with the disease, its differential diagnoses, and the interpretation of paraclinical assessments (imaging, CSF analysis, and evoked potentials) that can supplement the diagnostic process.

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References

1. Schumacher FA, Beeve GW, Kibler RF, et al. Problems of experimental trials of therapy in multiple sclerosis. Ann N Y Acad Sci 1965;122:552–568.
2. Poser CM, Paty DW, Scheinberg LC, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227–231.
3. Thompson AJ, Montalban X, Barkhof F, et al. Diagnostic criteria for primary progressive multiple sclerosis: a position paper. Ann Neurol 2000;47:831–835.
4. Gronseth GS, Ashman EJ. Practice parameter: the usefulness of

evoked potentials in identifying clinically silent lesions in patients with suspected multiple sclerosis (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2000;54:1720–1725.
5. Uthoff W. Untersuchungen über bei multiplen Herdsklerose vorkommenden Augenstorungen. Arch Psychiatr Nervenkrankh 1890;21:55–106, 303–410.
6. Barkhof F, Filippi M, Miller DH, et al. Comparison of MR imaging criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain 1997;120:2059–2069.
7. Tintoré M, Rovira A, Martínez M, et al. Isolated demyelinating syndromes: comparison of different MR imaging criteria to predict conversion to clinically definite multiple sclerosis. Am J Neuroradiol 2000;21:702–706.
8. Fazekas F, Offenbacher H, Fuchs S, et al. Criteria for an increased specificity of MRI interpretation in elderly subjects with suspected multiple sclerosis. Neurology 1988;38:1822–1825.
9. Offenbacher H, Fazekas F, Schmidt R, et al. Assessment of MRI criteria for a diagnosis of MS. Neurology 1993;43:905–909.
10. Paty DW, Oger JJ, Kastrukoff LF, et al. MRI in the diagnosis of MS: a prospective study with comparison of clinical evaluation, evoked potentials, oligoclonal banding and CT. Neurology 1988;38:180–185.
11. Thorpe JW, Kidd D, Kendall BE, et al. Spinal cord MRI using multi-array coils and fast spin echo. I. Technical aspects and findings in healthy adults. Neurology 1993;43:2625–2631.
12. Kidd C, Thorpe JW, Thompson AJ, et al. Spinal cord imaging MRI using multi-array coils and fast spin echo. II. Findings in multiple sclerosis. Neurology 1993;43:2632–2637.
13. Brex PA, O'Riordan JI, Miszkiel KA, et al. Multisequence MRI in clinically isolated syndromes and the early development of MS. Neurology 1999;53:1184–1190.
14. Andersson M, Alvarez-Cermeno J, Bernardi G, et al. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. J Neurol Neurosurg Psychiatr 1994;57:897–902.
15. Link H, Tibbling G. Principles of albumin and IgG analysis in neurological disorders. III. valuation of IgG synthesis within the central nervous system in multiple sclerosis. Scand J Clin Lab Invest 1977;37:397–401.
16. Halliday AM, editor. Evoked potentials in clinical testing, 2nd ed. London: Churchill Livingstone, 1993.
17. Dale RC, DeSousa C, Chong WK, et al. Acute disseminated encephalomyelitis and multiple sclerosis in children. A follow-up study to compare clinical and investigative findings on disease presentation. Brain 2000;123:2407–2422.
18. Gallagher HL, MacManus DG, Webb SL, Miller DH. A reproducible repositioning method for serial magnetic resonance imaging studies of the brain in treatment trials for multiple sclerosis. J Magnet Reson Imag 1997;7:439–441.
19. Matthews B. Differential diagnosis of multiple sclerosis and related disorders. In: Compston A, Ebers G, Lassmann H, et al, editors. McAlpine's multiple sclerosis, 3rd ed. London: Churchill Livingstone, 1998.
20. Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). Neurology 1999;53:1107–1114.
21. Phadke JG, Best PV. Atypical and clinically silent multiple sclerosis: a report of 12 cases discovered unexpectedly at necropsy. J Neurol Neurosurg Psychiatr 1983;46:414–420.
22. Brex FA, Miszkiel KA, O'Riordan JI, et al. Assessing the risk of early MS in patients with clinically isolated syndromes: the role of follow-up MRI. J Neurol Neurosurg Psychiatr 2001;70:390–393.

Introduction

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Therapeutic advances in ALS

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For patients, as well as for many physicians and scientists who have spent much of their careers trying to understand ALS, the data presented here represent renewed hope for the future. Only a few years ago, the idea that we would be discussing therapeutic advances in June 1995 would have been treated with some skepticism. Clearly, more progress is required before this work can have a significant impact on the progression of ALS. The mix of basic and clinical research presented in this Supplement represents a cross-section of the rapidly increasing momentum in the field of ALS investigation. There is an emerging consensus about pathophysiology—particularly regarding oxidative stress and excitotoxicity—and this offers great promise for the future.

The reviews by Leigh and Meldrum of the impact of excitotoxins in ALS serve to illustrate two points. First, the cascade of events leading to neuronal cell death is a complex process, involving many agents with potentially toxic effects, including free radicals, glutamate, and Ca^{2+} ions. Secondly, evidence obtained using many different approaches is accumulating, which supports a pathophysiologic role of excitotoxicity in this disease.

However, there remain many frustrations in ALS research, and Ludolph summarizes some of these in his article on animal models. The monosynaptic corticospinal tract is unique to primates and as this is the target system in ALS, the development of good animal models represents a substantial challenge. In addition, the biologic characteristics of the disease are not fully identified, leading to further difficulties. Nonetheless, the transgenic mouse with mutant SOD_1 represents an important forward step in examining pathophysiology and in screening potential therapeutic compounds.

In his article on the pharmacology of riluzole, Doble expands on the basic science of the first approved treatment for patients with ALS. The antiglutamate properties of riluzole appear to be mediated via four mechanisms: (1) inactivation of voltage-dependent sodium channels on glutamatergic nerve terminals; (2) inhibition of presynaptic release of glutamate; (3) noncompetitive blockade of postsynaptic NMDA channels; and (4) activation of a G protein-dependent pathway.

The results of the pivotal clinical study of riluzole, presented by Lacomblez, have been reported elsewhere.[1] This controlled trial is the first to show a significant impact on survival in ALS. In addition to confirmation of the safety and efficacy of riluzole (100 mg/day) seen in the first controlled study,[2] there were two other important outcomes. First, a dose response relationship was established, with 100 mg of riluzole being the dose of choice. Second, the trend for a difference in response between bulbar and limb onset patients noted in the earlier trial was not confirmed. Overall, the percentage differences in survival may appear small but, for the treated group, the relative risk of death or tracheostomy ratios at 12 months and at 18 months must be viewed as very positive outcomes.

In the final article, Hugon considers future approaches to the treatment of ALS. Thanks to the convergence of results from many different avenues of research, such speculation is now founded as much on experience as it is on hope. The biological processes leading to ALS are complex and possibly interdependent. It is not surprising, therefore, to expect that combinations of some or all of the following types of agents will be the best treatment options for the future:

- anti-excitotoxics
- neurotrophic factors
- free radical scavengers/anti-oxidants
- novel calcium channel blockers
- anti-apoptotic therapy
- gene therapy.

In conclusion, it is true to say that now, perhaps for the first time, scientists and clinicians alike have a new and genuine optimism about tackling this devastating disease. There are still many unanswered questions, and more basic science and well-controlled clinical studies are required before the current promise can be translated into tangible benefits for the thousands of patients, and their families, affected by ALS and the motor neuron diseases in general. However, there is now good reason to believe that we can build on these recent advances and look to a future where ALS will truly be a treatable disease.

References

1. Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V, for the Amyotrophic Lateral Sclerosis/Riluzole Study Group II. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Lancet 1996;347:1425–1431.
2. Bensimon G, Lacomblez L, Meininger V, for the ALS/Riluzole Study Group. A controlled trial of riluzole in amyotrophic lateral sclerosis. N Engl J Med 1994;330:585–591.

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Review Article

Medical Progress

MULTIPLE SCLEROSIS

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MORE than 100 years has passed since Charcot, Carswell, Cruveilhier, and others described the clinical and pathological characteristics of multiple sclerosis.[1] This enigmatic, relapsing, and often eventually progressive disorder of the white matter of the central nervous system continues to challenge investigators trying to understand the pathogenesis of the disease and prevent its progression.[2] There are 250,000 to 350,000 patients with multiple sclerosis in the United States.[3] Multiple sclerosis typically begins in early adulthood and has a variable prognosis. Fifty percent of patients will need help walking within 15 years after the onset of disease.[4] Advanced magnetic resonance imaging (MRI) and spectroscopy may allow clinicians to follow the pathological progression of the disease and monitor the response to treatment. Recent progress has occurred in understanding the cause, the genetic components, and the pathologic process of multiple sclerosis. The short-term clinical and MRI manifestations of disease activity have been reduced by new therapies, although the degree of presumed long-term benefit from these treatments will require further study.

CLINICAL COURSE AND DIAGNOSIS

A patient's presenting symptoms and the temporal evolution of the clinical findings may suggest the correct diagnosis. In relapsing–remitting multiple sclerosis — the type present in 80 percent of patients — symptoms and signs typically evolve over a period of several days, stabilize, and then often improve, spontaneously or in response to corticosteroids, within weeks. Relapsing–remitting multiple sclerosis typically begins in the second or third decade of life and has a female predominance of approximately 2:1. The tendency for corticosteroids to speed recovery from relapses often diminishes with time. Persistent signs of central nervous system dysfunction may develop after a relapse, and the disease may progress between relapses (secondary progressive multiple sclerosis). Twenty percent of affected patients have primary progressive multiple sclerosis, which is characterized by a gradually progressive clinical course and a similar incidence among men and women.

Relapsing–remitting multiple sclerosis typically starts with sensory disturbances, unilateral optic neuritis, diplopia (internuclear ophthalmoplegia), Lhermitte's sign (trunk and limb paresthesias evoked by neck flexion), limb weakness, clumsiness, gait ataxia, and neurogenic bladder and bowel symptoms. Many patients describe fatigue that is worse in the afternoon and is accompanied by physiologic increases in body temperature. The onset of symptoms post partum and symptomatic worsening with increases in body temperature (Uhthoff's symptom) and pseudoexacerbations with fever suggest the diagnosis. Some patients have recurring, brief, stereotypical phenomena (paroxysmal pain or paresthesias, trigeminal neuralgia, episodic clumsiness or dysarthria, and tonic limb posturing) that are highly suggestive of multiple sclerosis.

Prominent cortical signs (aphasia, apraxia, recurrent seizures, visual-field loss, and early dementia) and extrapyramidal phenomena (chorea and rigidity) only rarely dominate the clinical picture. Eventually, cognitive impairment, depression, emotional lability, dysarthria, dysphagia, vertigo, progressive quadriparesis and sensory loss, ataxic tremors, pain, sexual dysfunction, spasticity, and other manifestations of central nervous system dysfunction may become troublesome. Patients who have primary progressive multiple sclerosis often present with a slowly evolving upper-motor-neuron syndrome of the legs ("chronic progressive myelopathy"). Typically, this variant worsens gradually, and quadriparesis, cognitive decline, visual loss, brain-stem syndromes, and cerebellar, bowel, bladder, and sexual dysfunction may develop.

The diagnosis is based on established clinical and, when necessary, laboratory criteria.[5] Advances in cerebrospinal fluid analysis and MRI, in particular, have simplified the diagnostic process (Fig. 1).[6] The relapsing forms are considered clinically definite when neurologic dysfunction becomes "disseminated in space and time." Primary progressive multiple sclerosis may be suggested clinically by a progressive course that lasts longer than six months, but laboratory studies to obtain supportive evidence and efforts to exclude

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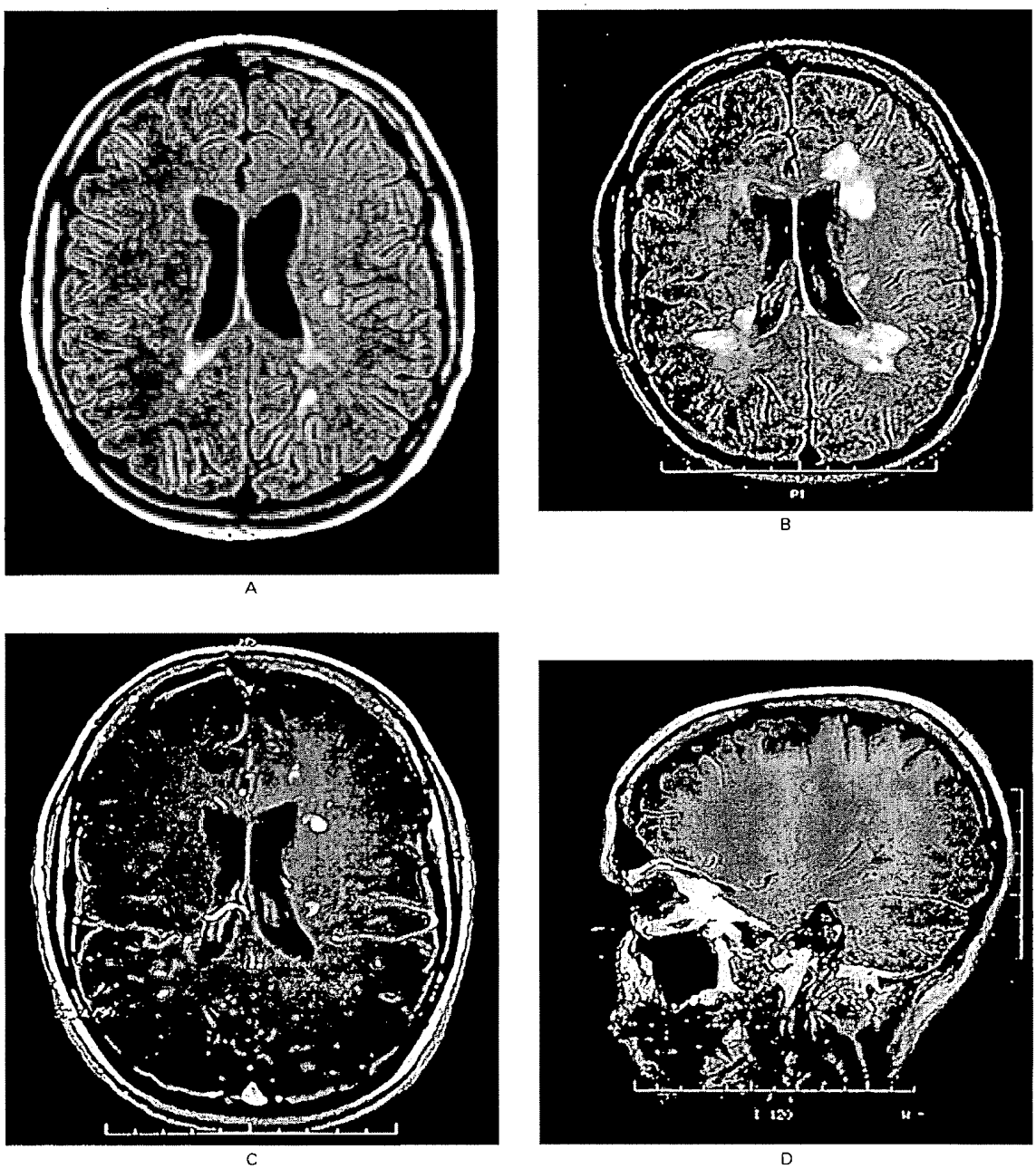


Figure 1. MRI Scans of the Brain of a 25-Year-Old Woman with Relapsing–Remitting Multiple Sclerosis.

An axial FLAIR (fluid-attenuated inversion recovery) image shows multiple ovoid and confluent hyperintense lesions in the periventricular white matter (Panel A). Nine months later, the number and size of the lesions have substantially increased (Panel B). After the administration of gadolinium, many of the lesions demonstrate ring or peripheral enhancement, indicating the breakdown of the blood–brain barrier (Panel C). In Panel D, a parasagittal T_1-weighted MRI scan shows multiple regions in which the signal is diminished (referred to as "black holes") in the periventricular white matter and corpus callosum. These regions correspond to the chronic lesions of multiple sclerosis.

other, potentially treatable illnesses are advised; for example, structural or metabolic myelopathy can be identified by appropriate laboratory studies, including spinal MRI (Table 1). On MRI, findings of multifocal lesions of various ages, especially those involving the periventricular white matter, brain stem, cerebellum, and spinal cord white matter, support the clinical impression. The presence of gadolinium-enhancing lesions on MRI indicates current sites of presumed inflammatory demyelination (active lesions).

When there is diagnostic uncertainty, repeated MRI after several months may provide evidence that the lesions are "disseminated in time." Cerebrospinal fluid analysis often shows increased intrathecal synthesis of immunoglobulins of restricted specificity (oligoclonal bands may be present, or the synthesis of IgG may be increased), with moderate lymphocytic pleocytosis (almost invariably there are fewer than 50 mononuclear cells). Physiologic evidence of subclinical dysfunction of the optic nerves and spinal cord (changes in visual evoked responses and somatosensory evoked potentials) may provide support for the conclusion that there is "dissemination in space."[7] Therefore, spinal MRI and evoked-potential testing may provide evidence of a second lesion that can confirm the diagnosis. Abnormalities detected by testing of somatosensory evoked potentials and spinal MRI may clarify the diagnosis in patients with optic neuritis alone or isolated brain-stem abnormalities and in those suspected of having unifocal cerebral multiple sclerosis on the basis of MRI. If positive, abnormalities detected by tests of visual evoked responses may support the diagnosis of multiple sclerosis in patients with isolated brain-stem or spinal cord lesions.

The course of multiple sclerosis in an individual patient is largely unpredictable. Patients who have a so-called clinically isolated syndrome (e.g., optic neuritis, brain-stem dysfunction, or incomplete transverse myelitis) as their first event have a greater risk of both recurrent events (thereby confirming the diagnosis of clinically definite multiple sclerosis) and disability within a decade if changes are seen in clinically asymptomatic regions on MRI of the brain.[8] The presence of oligoclonal bands in cerebrospinal fluid slightly increases the risk of recurrent disease.[9]

Studies of the natural history of the disease have provided important prognostic information that is useful for counseling patients and planning clinical trials.[4,10,11] Ten percent of patients do well for more than 20 years and are thus considered to have benign multiple sclerosis. Approximately 70 percent will have secondary progression.[4] Frequent relapses in the first two years, a progressive course from the onset, male sex, and early, permanent motor or cerebellar findings are independently, but imperfectly, predictive of a more severe clinical course. Women and patients with predominantly sensory symptoms and optic neuritis have a more favorable prognosis. Life expectancy may be shortened slightly; in rare cases, patients with fulminant disease die within months after the onset of multiple sclerosis. Suicide remains a risk, even for young patients with mild symptoms.[12]

TABLE 1. DIFFERENTIAL DIAGNOSIS OF MULTIPLE SCLEROSIS.

Metabolic disorders
Disorders of B_{12} metabolism*
Leukodystrophies
Autoimmune diseases
Sjögren's syndrome, systemic lupus erythematosus, Behçet's disease, sarcoidosis, chronic inflammatory demyelinating polyradiculopathy associated with central nervous system demyelination, antiphospholipid-antibody syndrome
Infections†
HIV-associated myelopathy* and HTLV-1–associated myelopathy,* Lyme disease, meningovascular syphilis, Eales' disease
Vascular disorders
Spinal dural arteriovenous fistula*
Cavernous hemangiomata
Central nervous system vasculitis, including retinocochlear cerebral vasculitis
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
Genetic syndromes
Hereditary ataxias and hereditary paraplegias*
Leber's optic atrophy and other mitochondrial cytopathies
Lesions of the posterior fossa and spinal cord
Arnold–Chiari malformation, nonhereditary ataxias
Spondylotic and other myelopathies*
Psychiatric disorders
Conversion reaction, malingering
Neoplastic diseases
Spinal cord tumors,* central nervous system lymphoma
Paraneoplastic disorders
Variants of multiple sclerosis‡
Optic neuritis; isolated brain-stem syndromes; transverse myelitis; acute disseminated encephalomyelitis, Marburg disease; neuromyelitis optica

*This disorder or group of disorders is of particular relevance in the differential diagnosis of progressive myelopathy and primary progressive multiple sclerosis.

†HIV denotes human immunodeficiency virus, and HTLV-1 human T-cell lymphotropic virus type 1.

‡In many patients with these variants, clinically definite multiple sclerosis develops or the course is indistinguishable from that of multiple sclerosis.

EPIDEMIOLOGIC FEATURES

The prevalence of multiple sclerosis varies considerably around the world.[13] Kurtzke classified regions of the world according to prevalence: a low prevalence was considered less than 5 cases per 100,000 persons, an intermediate prevalence was 5 to 30 per 100,000 persons, and a high prevalence was more than 30 per 100,000 persons.[14] The prevalence is highest in northern Europe, southern Australia, and the middle part of North America. There has been

a trend toward an increasing prevalence and incidence, particularly in southern Europe.[15,16] Even in areas with uniform methods of ascertainment and high prevalence, such as Olmsted County, Minnesota, the incidence has increased from 2 to 6 per 100,000 during the past century.[17] However, the incidence has actually declined in some,[18,19] but not all,[20] areas of northern Europe. Stable or declining rates have been reported most often in regions with high prevalence and incidence. The extent to which the observed increases in incidence are explained by an enhanced awareness of the disease and improved diagnostic techniques is uncertain. There is a large reservoir of mild cases, the recognition of which may depend heavily on the zeal and resources of the investigator.

The reasons for the variation in the prevalence and incidence of multiple sclerosis worldwide are not understood. Environmental and genetic explanations have been offered, and both factors probably have a role. The occurrence of rapid shifts in the incidence of multiple sclerosis, if not artifactual, is an argument for an environmental influence, as is the equivocal, but suggestive, evidence of the clustering of cases in terms of both geography and time and of epidemics, especially on the Faroe Islands.[21] The apparent change in the frequency of multiple sclerosis among people[22,23] and their offspring[24] who migrate to and from high-prevalence areas is another factor that has been presented to support the existence of an environmental factor. However, each of these relations has potential confounders that preclude the drawing of a definite conclusion regarding the importance of environmental factors.[25] The nature of putative environmental factors remains unclear in numerous case–control studies. Studies that show that the incidence of multiple sclerosis among the adopted children of patients with multiple sclerosis is not higher than expected seem to argue against the possibility that a transmissible factor is primarily responsible for the increased risk of the disease among relatives and instead suggest that genetic factors may be responsible.[26]

GENETIC FACTORS

Evidence that genetic factors have a substantial effect on susceptibility to multiple sclerosis is unequivocal. The concordance rate of 31 percent among monozygotic twins is approximately six times the rate among dizygotic twins (5 percent).[27] The absolute risk of the disease in a first-degree relative of a patient with multiple sclerosis is less than 5 percent; however, the risk in such relatives is 20 to 40 times the risk in the general population.[28] Since 1973, it has been recognized that the presence of the HLA-DR2 allele substantially increases the risk of multiple sclerosis.[29] This effect has been found in all populations, with the exception of that in Sardinia.[30] The magnitude of the relative risk depends on the frequency of the HLA-DR2 allele in the general population. Given the high frequency of this allele in the population, the risk attributable to the HLA-DR2 allele is considerable. Populations with a high frequency of the allele (e.g., those in Scotland) have the highest risk of multiple sclerosis.

The mode of transmission of genetic susceptibility to multiple sclerosis is complex. Most cases are sporadic, despite the clear excess risk among the relatives of patients. Investigators have used the usual genetic approaches to identify genes associated with an increased risk of multiple sclerosis.

Studies of candidate genes have targeted individual genes with microsatellite markers with use of association and linkage strategies. For some genetic regions, such as the HLA region on chromosome 6, it has been difficult to identify the specific polymorphism that predisposes persons to the disease, given the high degree of linkage disequilibrium at that locus. Candidate-gene studies were followed by four studies in which the entire genome was scanned.[31-34] Regions of interest have been identified, although none have been linked to the disease with certainty. Considering the rather large number of patients evaluated in such studies, one might conclude tentatively that no single gene, except possibly those for HLA antigens,[35] exerts a strong effect.

Further refinement of the linkage map is in progress.[36] Whether this approach will prove powerful enough to identify genes with a relatively weak effect is difficult to predict. To enhance the detection of genes with a weak effect, investigators have begun to use strategies involving linkage-disequilibrium mapping and transmission-disequilibrium testing. In these approaches, putative causative alleles or marker alleles and haplotypes are assessed to determine whether they are associated with the disease at a population level or whether they are associated with a higher-than-expected rate of transmission of disease from heterozygous parents to their children. This effort will involve a major expenditure of resources to achieve genome-wide coverage. The development of novel analytic techniques for these types of genetic data sets makes such an undertaking feasible.[37]

The severity and course of multiple sclerosis may also be influenced by genetic factors. Epidemiologic evidence to support this premise comes from studies examining the rate of concordance for measures that describe and quantitate variations in the course of disease, including the age at onset, the proportion of patients in whom the disease progresses, and the extent of disability over time.[38] HLA-DR and DQ polymorphisms are not associated with the course and severity of multiple sclerosis, despite their substantial contribution to disease susceptibility.[39] Recently, variants of the interleukin-1β–receptor and interleukin-1–receptor antagonist genes,[40] immunoglobulin Fc receptor genes,[41] and apolipoprotein E gene[42] have been associated with the course of the disease, but these findings await confirmation.

PATHOLOGICAL FEATURES AND PATHOGENESIS

Multiple sclerosis is generally believed to be an immune-mediated disorder that occurs in genetically susceptible people (Fig. 2).[43] However, the sequence of events that initiates the disease remains largely unknown. Given the considerable clinical, genetic, MRI, and pathological heterogeneity of multiple sclerosis, perhaps more than one pathogenetic mechanism contributes to tissue injury. This possibility has therapeutic implications, because more than one approach to treatment may be required to treat this disease effectively.

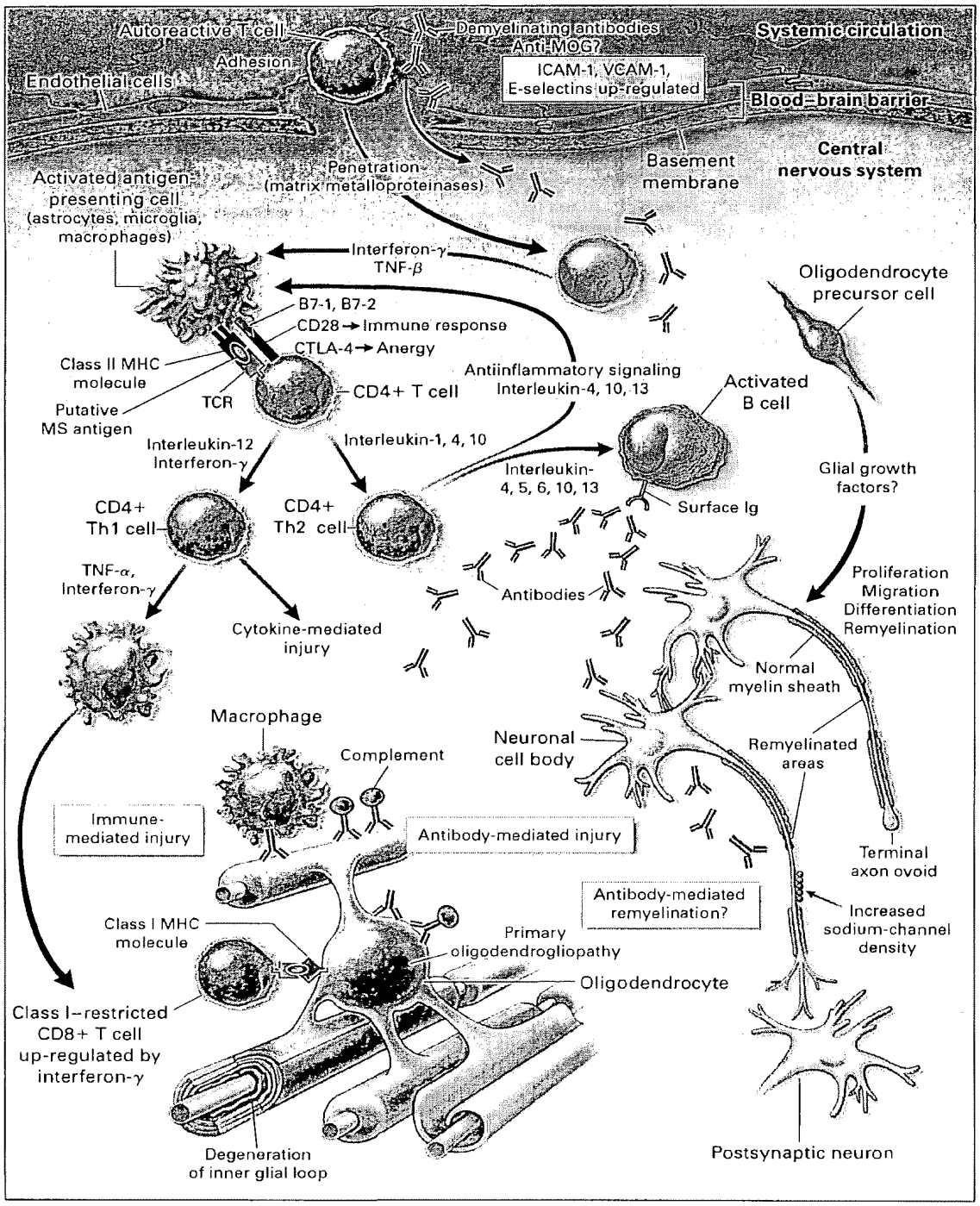
The pathological hallmark of chronic multiple sclerosis is the demyelinated plaque, which consists of a well-demarcated hypocellular area characterized by the loss of myelin, relative preservation of axons, and the formation of astrocytic scars (Fig. 3). Lesions have a predilection for the optic nerves, periventricular white matter, brain stem, cerebellum, and spinal cord white matter, and they often surround one or several medium-sized vessels. Although the lesions are usually round or oval, they often have finger-like extensions along the path of small or medium-sized blood vessels (Dawson's fingers). Inflammatory cells are typically perivascular in location, but they may diffusely infiltrate the parenchyma. The composition of the inflammatory infiltrate varies depending on the stage of demyelinating activity. In general, it is composed of lymphocytes and macrophages; the latter predominate in active lesions.

For meaningful conclusions to be drawn regarding the earliest immunologic and molecular events contributing to the formation of lesions, only actively demyelinating plaques should be considered. Identifying myelin-degradation products in macrophages is the most reliable method of identifying active lesions (Fig. 4).[44] When stringent criteria are used to define lesional activity, the frequency of active plaques in patients with chronic multiple sclerosis is extremely low. Although remyelination is minimal in lesions associated with chronic multiple sclerosis, plaques in acute and early multiple sclerosis may have extensive remyelination (referred to as shadow plaques) (Fig. 5). Furthermore, the lesions of chronic multiple sclerosis reportedly contain substantial numbers of oligodendrocyte precursor cells.[45] Thus, central nervous system myelin can be repaired, and mechanisms that promote endogenous remyelination may represent a feasible therapeutic strategy.

Early symptoms of multiple sclerosis are widely believed to result from axonal demyelination, which leads to the slowing or blockade of conduction. The regression of symptoms has been attributed to the resolution of inflammatory edema and to partial remyelination. However, inflammatory cytokines may inhibit axonal function, and the recovery of function

Figure 2 (facing page). Possible Mechanisms of Injury and Repair in Multiple Sclerosis.

Genetic and environmental factors (including viral infection, bacterial lipopolysaccharides, superantigens, reactive metabolites, and metabolic stress) may facilitate the movement of autoreactive T cells and demyelinating antibodies from the systemic circulation into the central nervous system through disruption of the blood–brain barrier. In the central nervous system, local factors (including viral infection and metabolic stress) may up-regulate the expression of endothelial adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), vascular-cell adhesion molecule 1 (VCAM-1), and E-selectin, further facilitating the entry of T cells into the central nervous system. Proteases, including matrix metalloproteinases, may further enhance the migration of autoreactive immune cells by degrading extracellular-matrix macromolecules. Proinflammatory cytokines released by activated T cells, such as interferon-γ and tumor necrosis factor β (TNF-β), may up-regulate the expression of cell-surface molecules on neighboring lymphocytes and antigen-presenting cells. Binding of putative multiple sclerosis (MS) antigens, such as myelin basic protein, myelin-associated glycoprotein, myelin oligodendrocyte glycoprotein (MOG), proteolipid protein, αB-crystallin, phosphodiesterases, and S-100 protein, by the trimolecular complex — the T-cell receptor (TCR) and class II major-histocompatibility-complex (MHC) molecules on antigen-presenting cells — may trigger either an enhanced immune response against the bound antigen or anergy, depending on the type of signaling that results from interactions with surface costimulatory molecules (e.g., CD28 and CTLA-4) and their ligands (e.g., B7-1 and B7-2). Down-regulation of the immune response (anergy) may result in the release of antiinflammatory cytokines (interleukin-1, interleukin-4, and interleukin-10) from CD4+ T cells, leading to the proliferation of antiinflammatory CD4+ type 2 helper T (Th2) cells. Th2 cells may send antiinflammatory signals to the activated antigen-presenting cells and stimulate pathologic or repair-enhancing antibody-producing B cells. Alternatively, if antigen processing results in an enhanced immune response, proinflammatory cytokines (e.g., interleukin-12 and interferon-γ) may trigger a cascade of events, resulting in the proliferation of proinflammatory CD4+ type 1 helper T (Th1) cells and ultimately in immune-mediated injury to myelin and oligodendrocytes. Multiple mechanisms of immune-mediated injury of myelin have been postulated: cytokine-mediated injury of oligodendrocytes and myelin; digestion of surface myelin antigens by macrophages, including binding of antibodies against myelin and oligodendrocytes (i.e., antibody-dependent cytotoxicity); complement-mediated injury; and direct injury of oligodendrocytes by CD4+ and CD8+ T cells. This injury to the myelin membrane results in denuded axons that are no longer able to transmit action potentials efficiently within the central nervous system (loss of saltatory conduction). This slowing or blocking of the action potential results in the production of neurologic symptoms. The exposed axon segments may be susceptible to further injury from soluble mediators of injury (including cytokines, chemokines, complement, and proteases), resulting in irreversible axonal injury (such as axonal transection and terminal axon ovoids). There are several possible mechanisms of repair of the myelin membrane, including resolution of the inflammatory response followed by spontaneous remyelination, spread of sodium channels from the nodes of Ranvier to cover denuded axon segments and restore conduction, antibody-mediated remyelination, and remyelination resulting from the proliferation, migration, and differentiation of resident oligodendrocyte precursor cells. Adapted from a drawing by the Mayo Foundation.



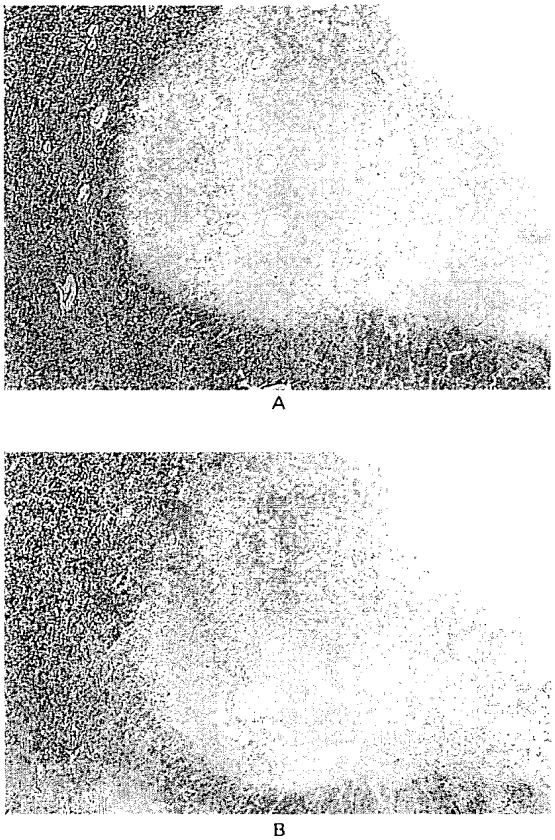


Figure 3. Photomicrographs of a Chronic Multiple Sclerosis Plaque.

In Panel A, a well-demarcated hypocellular region of myelin loss is evident in the periventricular white matter (luxol fast blue and periodic acid–Schiff myelin stain, ×15). In Panel B, neurofilament staining for axons in the same lesion demonstrates a reduction in axonal density (×15).

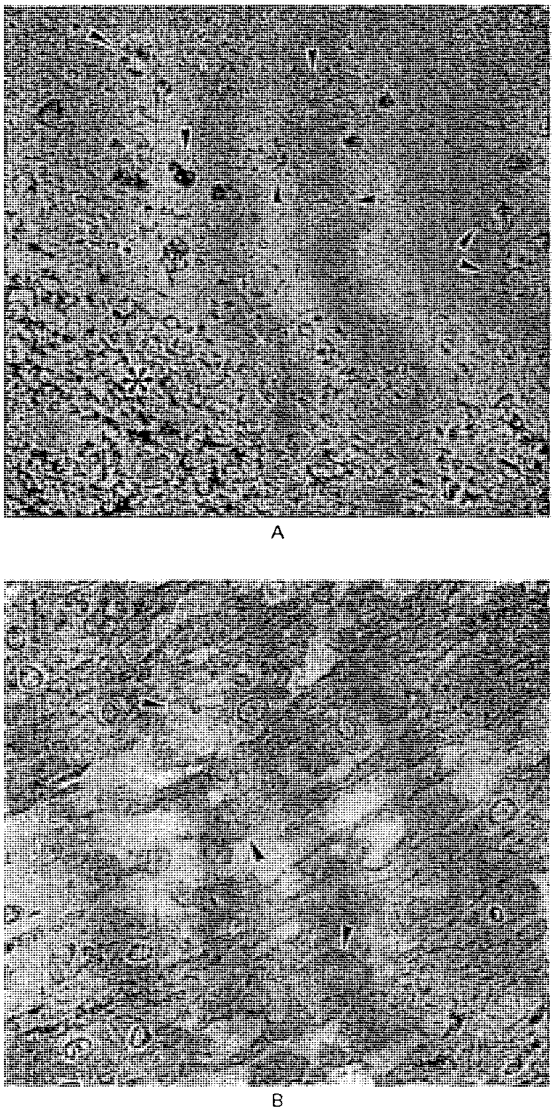


Figure 4. Photomicrographs of an Actively Demyelinating Multiple Sclerosis Lesion (Immunocytochemical Staining of Myelin Oligodendrocyte Glycoprotein [Brown] with Hematoxylin Counterstaining of Nuclei [Blue]).

In Panel A, at the active edge of a multiple sclerosis lesion (indicated by the asterisk), the products of myelin degradation are present in numerous macrophages (arrowheads) (×100). In Panel B (×100), macrophages containing myelin debris (arrowheads) are interdigitated with degenerating myelin sheaths.

may result from the redistribution of sodium channels across segments of demyelinated axons.[46,47] Irreversible axonal injury, gliotic scarring, and exhaustion of the oligodendrocyte progenitor pool may result from repeated episodes of disease activity and lead to progressive loss of neurologic function. Axonal injury may occur not only in the late phases of multiple sclerosis but also after early episodes of inflammatory demyelination.[48-50] The pathogenesis of this early axonal injury is still unclear.

Experimental in vitro and in vivo models of inflammatory demyelination suggest that diverse disease processes, including autoimmunity and viral infection,

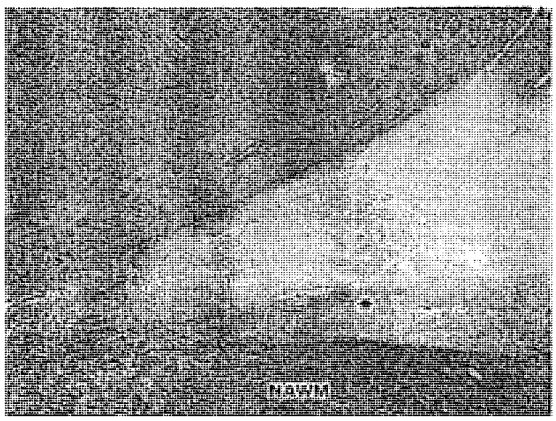


Figure 5. Remyelination in a Lesion Associated with Chronic Multiple Sclerosis.

The area stained pale blue (indicated by the asterisk) represents a region of partial remyelination (a shadow plaque) along the periventricular edge of a lesion in a patient with chronic multiple sclerosis (luxol fast blue and periodic acid–Schiff myelin stain, ×15). NAWM denotes normal-appearing white matter.

may induce multiple sclerosis–like inflammatory demyelinated plaques. Activated CD4+ T cells specific for one or more self antigens are believed to adhere to the luminal surface of endothelial cells in central nervous system venules and migrate into the central nervous system at the time of disruption of the blood–brain barrier. This process is followed by an amplification of the immune response after the recognition of target antigens on antigen-presenting cells. The existence of T cells that are reactive to several putative self myelin and non-myelin "multiple sclerosis antigens," including myelin basic protein, myelin-associated glycoprotein, myelin oligodendrocyte glycoprotein, proteolipid protein, αB-crystallin, phosphodiesterases, and S-100 protein, has been proposed.[51-53] Additional amplification factors including autoantibodies or cytokines may also be necessary to produce the demyelinated plaque[2,54] (Fig. 2).

Antibodies against antigens located on the surface of the myelin sheath or oligodendrocyte can cause demyelination directly, possibly through the activation of complement, leading to complement-mediated cytolysis.[55] These antibodies may gain access to the central nervous system through the disruption of the blood–brain barrier as a consequence of a T-cell–initiated inflammatory response. The existence of antibody-mediated demyelination is supported in part by the observation that demyelination was augmented by the administration of antibody specific for myelin oligodendrocyte glycoprotein to rats with experimentally induced allergic encephalomyelitis[56] (the glycoprotein is present on the outer lamellae of the myelin sheath). Antibodies against both myelin oligodendrocyte glycoprotein and myelin basic protein can be found in the brains of patients with multiple sclerosis.[57] Deposits of immunoglobulin and activated complement may be present in multiple sclerosis lesions in which myelin is being degraded.[58] Taken together, these observations suggest that an antibody-mediated process may have an important role in the pathogenesis of multiple sclerosis.

Other factors may also help degrade myelin and damage oligodendrocytes. Activated macrophages and microglial cells may mediate such activity by producing proinflammatory cytokines (such as tumor necrosis factor α and interferon-γ), generating reactive oxygen or nitrogen species, producing excitatory amino acids, activating complement components, or releasing proteolytic and lipolytic enzymes. Other factors potentially toxic to oligodendroglial cells include soluble T-cell products (such as perforin), the interaction of Fas antigen with Fas ligand, cytotoxicity mediated by the interaction of CD8+ T cells with class I major-histocompatibility-complex (MHC) antigens on antigen-presenting cells, and persistent viral infection.[54] Human herpesvirus type 6 can cause a condition that mimics multiple sclerosis[59] and appears in oligodendrocytes within multiple sclerosis tissue in some patients, but not in control tissue.[60] A direct causal link, however, remains to be confirmed. In one study, *Chlamydia pneumoniae* was isolated from 64 percent of patients with multiple sclerosis, as compared with 11 percent of control patients with other neurologic diseases, and it was detected in cerebrospinal fluid by a polymerase-chain-reaction assay in 97 percent of patients with multiple sclerosis, as compared with 18 percent of control patients.[61] These results have yet to be confirmed in other laboratories.[62]

Various pathogenic mechanisms may be involved in multiple sclerosis. There is an important degree of variability among patients in the structural and immunologic features of the lesions of multiple sclerosis.[63] The extent of survival of oligodendrocytes varies from patient to patient but is uniform within a given patient, suggesting that the focus of injury (myelin, mature oligodendrocyte, or progenitor cell) varies among patients.[49] Although most lesions are characterized by an inflammatory reaction, composed mainly of T lymphocytes and macrophages, diverse patterns of myelin destruction have been described.[64]

In some lesions, the presence of immunoglobulins and activated terminal complement components suggests that demyelinating antibodies have a pathogenic role. In others, a primary oligodendrocyte dystrophy manifested by the selective loss of myelin-associated glycoprotein and apoptosis of oligodendrocytes has been seen. Finally, in other cases, a small rim of necrotic oligodendrocytes has been found in the nor-

mal-appearing white matter adjacent to the active plaque edge. The patterns of demyelination were heterogeneous among patients, but homogeneous within active plaques from the same patient. Multiple sclerosis may therefore be a series of syndromes with different causes and pathogenic mechanisms (e.g., cellular-mediated immune injury, complement- and antibody-mediated injury, or primary oligodendroglial dystrophy). If confirmed, this possibility could lead to the identification of markers of the underlying pathologic processes that could be used to individualize treatment.

MRI and spectroscopy may be helpful in characterizing the underlying pathologic processes in multiple sclerosis.[6] There is consensus that T_2-weighted MRI reflects a broad spectrum of pathological changes, including inflammation, edema, demyelination, gliosis, and axonal loss. Changes in the number and volume of lesions on T_2-weighted MRI (referred to as the T_2-weighted lesion load) are sensitive but nonspecific indicators of disease activity and the response to treatment. New lesions and areas of gadolinium enhancement on T_1-weighted MRI suggest recent inflammatory demyelination with disruption of the blood–brain barrier (Fig. 1). Monitoring by means of serial MRI studies with gadolinium enhancement helps to identify agents that may be active against this early inflammatory stage of multiple sclerosis (e.g., corticosteroids, interferons, glatiramer acetate, and certain immunosuppressive agents).[65-69]

There is MRI and pathological evidence that the normal-appearing white matter is not normal in patients with multiple sclerosis.[70,71] Serial MRI studies of normal-appearing white matter may be useful to determine where abnormalities are likely to develop.[72] Findings of "black holes" on T_1-weighted images, changes in magnetization-transfer ratios (a measure of free and bound water, which is an indication of the degree of structural disruption) (Fig. 1), and serial decreases in the volume of the brain and spinal cord (indicating atrophy) on imaging studies most likely correlate with both the loss of axons and the occurrence of extensive demyelination; these may ultimately be useful markers of the late, secondary degenerative phase of the illness. These measures, along with MRI spectroscopic markers of the number and function of neurons (e.g., the levels of *N*-acetyl aspartate), may eventually prove to be valid, objective surrogate measures of axonal abnormalities.

TREATMENT

Principles of Therapy

Patients with multiple sclerosis face enormous prognostic uncertainty, and they must become well informed about their illness. This is perhaps best accomplished with a multidisciplinary approach involving a neurologist, an allied health worker (e.g., nurse or a social worker) with expertise in multiple sclerosis, and information from national and local multiple sclerosis organizations. Treating physicians must continually assess the need for psychological support for patients and their families, since depression is common and the rate of suicide is relatively high in this population of patients.[12]

Physicians and patients need to distinguish clinical relapses from the transient worsening of symptoms that may accompany an increase in body temperature or fatigue. Patients should be reassured that findings of recent disease activity do not invariably indicate an unfavorable long-term prognosis and that pregnancy does not worsen the long-term outcome.[73] Patients should limit their exposure to viral illnesses because infections may trigger relapses.[74] Vaccinations may be safely administered to patients who may be at risk for influenza.[75] Because of reports that the hepatitis B virus vaccine may trigger multiple sclerosis, this vaccine should be administered only to persons at substantial risk of exposure to the virus — until the relative risks associated with vaccination are clarified by definitive, prospective studies that include MRI.

Relapses

Corticosteroids are often used to treat clinically significant relapses in an attempt to hasten recovery; for example, intravenous methylprednisolone may be given for five days, followed by an optional brief course of prednisone. There is no consensus about the optimal form, dose, route, or duration of corticosteroid therapy (Table 2). Other experimental strategies[78] have not proved to be better than corticosteroids. A post hoc analysis of the Optic Neuritis Treatment Trial suggested that prednisone might increase the risk of recurrent episodes of disease activity[79] and that early intervention with intravenous methylprednisolone and prednisone delayed the recurrence of neurologic events for two years.[80] These findings changed clinical practice: oral prednisone is now rarely used to treat acute optic neuritis.

A recent, double-blind, crossover trial demonstrated that a regimen of seven alternate-day plasma exchanges was followed by substantial clinical improvement in approximately 40 percent of patients who had catastrophic episodes of inflammatory demyelination that were unresponsive to corticosteroids.[81] These results require confirmation.

The optimal treatment of patients after a first clinical episode of possible multiple sclerosis remains uncertain. As discussed earlier, the risk of recurrence and the extent of disability can to some extent be predicted by the findings on MRI of the brain at the time of the first clinical episode.[8] Two recently completed phase 3 trials[82,83] suggest that treatment with interferon beta-1a may delay the development of a second, diagnosis-defining bout (clinically definite multiple sclerosis). In this issue of the *Journal*, Jacobs et al.[83] report that early treatment with interferon beta-1a

TABLE 2. CURRENT TREATMENTS FOR MULTIPLE SCLEROSIS.

TYPE OF MULTIPLE SCLEROSIS OR RELAPSE	AGENT	DOSE	KNOWN OR POSSIBLE BENEFITS OF TREATMENT	UNKNOWN EFFECTS OR ASPECTS OF TREATMENT
Relapsing–remitting	Interferon beta-1b (Betaseron)	8 million IU subcutaneously every other day	Reduces rate of clinical relapse Reduces the development of new lesions on MRI Delays the increase in the volume of lesions on MRI	Ability to delay progression of disability Duration and clinical significance of benefit Mechanism of action Most effective dose and route of administration Frequency and clinical significance of the formation of neutralizing antibodies
	Interferon beta-1a (Avonex)	30 μg intramuscularly once weekly	Reduces rate of clinical relapse May delay progression of disability Reduces the development of new lesions on MRI Delays the increase in the volume of lesions on MRI	Whether the effect on disability is clinically meaningful and sustained Duration and clinical significance of benefit Mechanisms of action Most effective dose and route of administration Frequency and clinical significance of the formation of neutralizing antibodies
	High-dose interferon beta-1a (Rebif)*	22 or 44 μg subcutaneously every other day	Possible dose-related benefit in patients with more severe disabilities	
	Glatiramer acetate (Copaxone)	20 μg subcutaneously daily	Reduces rate of clinical relapse Moderately reduces the development of new lesions on MRI	Effect on the progression of disability Duration and clinical significance of benefit Mechanism of action Most effective dose and route of administration
	Immune globulin	0.15–0.2 g/kg of body weight intravenously monthly for 2 yr	Reduces rate of clinical relapse May delay progression of disability	Whether progression of disability is actually delayed, as measured by a second evaluation in 3 mo Effect on the number and volume of lesions, as assessed by MRI Duration and clinical significance of benefit Mechanism of action Most effective dose and route of administration
Secondary progressive	Interferon beta-1b (Betaferon)	8 million IU subcutaneously every other day	Reduces rate of clinical relapse May reduce progression of disability regardless of relapse status (recent or current)† Delays the increase in the volume of lesions on MRI	Whether progression of disability is actually delayed, and if so, for how long and to what effect Mechanism of action Most effective dose and route of administration Frequency and clinical significance of the formation of neutralizing antibodies
	Mitoxantrone hydrochloride	5 or 12 mg/m² of body-surface area intravenously every 3 mo for 2 yr	Reduces rate of clinical relapse Delays progression of disability Reduces activity evident on MRI	Duration of benefit Most effective dose Dose-dependent risk of cardiac toxicity
Primary progressive	None			
Acute relapses	Corticosteroids	Various doses (see text)	Hastens clinical recovery Transiently restores blood–brain barrier on MRI	Duration and clinical significance of benefit Effect on progression of disability Mechanism of action Most effective agent, dose, and route of administration Why responsiveness to corticosteroids declines over time
	Plasma exchange	Seven exchanges of one plasma volume on alternate days	Enhances recovery of relapse-related neurologic deficits in patients with no response to high-dose corticosteroids	Effect on recurrent disease Duration of effect Mechanism of action

*This formulation is only available in Canada and Europe.

†This benefit has been observed in one of two studies.[76,77]

delayed the development of clinical and MRI evidence of recurrent disease in patients with a first demyelinating central nervous system event. This is an expected finding, given the published evidence that interferon beta reduces clinical relapses and changes on MRI scans. This report may influence patients' and physicians' decisions regarding the timing of interferon therapy, although the inconvenience, treatment-related side effects, cost, and lack of evidence of an important long-term benefit of interferon beta will deter others from starting treatment early in the disease course. The relations among inflammatory-mediated demyelination, axonal injury, and clinical disability remain to be clarified. There is a pressing need to determine whether the currently approved, partially effective immunomodulatory therapies re-

duce the degree or delay the development of disability in patients with clinically isolated demyelinating syndromes and definite multiple sclerosis.

Relapsing Multiple Sclerosis

The first of several convincing trials demonstrated that interferon beta-1b (Betaseron, Berlex Laboratories) reduced the frequency of relapse by approximately 30 percent.[65,66,84] There was also a trend toward a delay in the progression of disability, but this finding did not reach statistical significance. Interferon beta-1a (Avonex, Biogen) and glatiramer acetate (Copaxone, Teva Pharmaceutical Industries)[85,86] were subsequently found to reduce the frequency of relapse. Interferon beta-1a may delay the progression of disability in patients with minor disability who have a relapsing form of multiple sclerosis.[67,87,88]

Each of these agents has a number of immune-mediating activities; the specific mechanisms of action of these agents in multiple sclerosis are incompletely understood. The interferons reduce the proliferation of T cells and the production of tumor necrosis factor α, decrease antigen presentation, alter cytokine production to favor ones governed by type 2 helper T (Th2) cells, increase the secretion of interleukin-10, and reduce the passage of immune cells across the blood–brain barrier by means of their effects on adhesion molecules, chemokines, and proteases. Glatiramer acetate, formerly known as copolymer-1, is a mixture of synthetic polypeptides containing the L-amino acids glutamic acid, alanine, lysine, and tyrosine. Glatiramer acetate may promote the proliferation of Th2 cytokines; compete with myelin basic protein for presentation on MHC class II molecules, thereby inhibiting antigen-specific T-cell activation (Fig. 2); alter the function of macrophages; and induce antigen-specific suppressor T cells.

All these drugs reduce the development of new, gadolinium-enhancing lesions on MRI with variable effectiveness. All three agents are approved by the Food and Drug Administration (FDA) and are used widely. A higher-dose formulation of interferon beta-1a (Rebif, Ares Serono International) has yet to be approved for use in the United States but is licensed for use in Canada and Europe.[68] These agents must be administered parenterally, are expensive (each costs approximately $10,000 per year in the United States), and have variable adverse effects. Their long-term effectiveness has not been established, and studies are now addressing the cost effectiveness of these agents.[89] Interferon beta-1a and interferon beta-1b may induce the formation of neutralizing antibodies, especially during the first 18 months of treatment. The relevance of neutralizing antibodies, particularly with regard to the level that is clinically significant, is uncertain. There is concern that high titers of neutralizing antibodies may decrease or abrogate the biologic activity of interferon beta. It may be advisable to test patients for neutralizing antibodies if they have no response to interferon beta, although practice guidelines with respect to the interpretation of these tests are not yet available.

Opinions vary on when to initiate treatment with interferon beta and glatiramer acetate. The practice directive of the National Multiple Sclerosis Society states that these agents should be considered in patients with relapsing–remitting multiple sclerosis who have had recent relapses.[90] Neurologists who initiate treatment when the diagnosis of relapsing–remitting multiple sclerosis is established, or shortly thereafter, believe that these drugs are maximally effective against the early inflammatory phase of the disease. They reason that treatment may limit irreversible axonal injury and delay late deterioration; this hypothesis is based in part on evidence from biopsy studies showing that axonal injury can occur in acute or severe multiple sclerosis.[48] Other neurologists delay treatment until there is a history of recurrent relapses over a more prolonged period, for a number of reasons. Patients may have a benign early course.[91]

Data on the long-term efficacy and safety of these agents are not available. Although axonal injury may occur early, the frequency of early axonal injury is unknown. The formation of neutralizing antibodies may render interferon beta inactive, leaving the patient without this treatment option later in the clinical course. There is no evidence that these agents reduce such injury. The enthusiasm for these treatments, whether started immediately after the diagnosis is made or sometime later, must be tempered by the disappointing reality that most patients continue to have relapses during treatment and ultimately become increasingly disabled.

Patients frequently have firm opinions about the timing and choice of treatment. Given that there are no long-term studies (e.g., ones lasting longer than five years) confirming that any of the agents delay the progression of disability and that there have been no phase 3 comparative studies clarifying which agent is most effective, the treating physician must consider the patient's individual risk of clinically significant early disability and the patient's desire to start or delay treatment. Many North American neurologists initiate treatment after repeated relapses, particularly if the patient's clinical recovery is incomplete. The choice of the specific agent remains highly dependent on the specialist's opinion of its relative potency and the patient's anticipated tolerance of treatment-related side effects. Glatiramer acetate is generally well tolerated and may be most effective for mildly disabled patients with a recent diagnosis of multiple sclerosis who wish to start treatment early in the course of the illness.

Some multiple sclerosis specialists believe that the published evidence favors interferon beta, although the side effects are generally more troublesome than

those of glatiramer acetate. The evidence of a dose–response for interferon beta[66,68,92,93] may influence the treating physician in the United States to choose interferon beta-1b, which delivers a higher cumulative weekly dose of interferon, rather than interferon beta-1a. (A high-dose formulation of interferon beta-1a [Rebif] is available in Canada and Europe.) Higher doses, however, may be accompanied by more frequent side effects and an increased risk of the formation of neutralizing antibodies. If these factors are considered paramount, the treating physician may choose a lower dose of weekly interferon beta-1a.

One placebo-controlled trial reported that intravenous immune globulin reduced the frequency of relapse.[94] These results have yet to be confirmed, and immune globulin is not widely used for this indication in North America.

Secondary Progressive Multiple Sclerosis

The indications for the treatment of secondary progressive multiple sclerosis are unclear. Many trials have reported a marginal benefit with various immunosuppressive therapies. A recent phase 3 European trial reported that interferon beta-1b reduced clinical and MRI evidence of disease activiry.[69,76] Treatment delayed the progression of disability regardless of whether relapses occurred before or after randomization, although the magnitude of the effect was moderate. It is not known whether this benefit in patients who were not having ongoing relapses results from an ability of interferon to interfere with the degenerative changes that presumably contribute to the clinical worsening that occurs in most patients after the first decade of the illness. Alternatively, this apparent benefit may reflect an ability of interferon to reduce inflammatory activity, whether manifested clinically or not (e.g., subclinical relapses).

Interferon beta-1b has been approved for use in secondary progressive multiple sclerosis in Europe and Canada. The results of two recently completed phase 3 trials[77,95,96] indicate that interferon beta-1b and interferon beta-1a may reduce the frequency of relapses and the evidence of disease activity on MRI only in patients who have continual clinical relapses. However, neither of these studies found that treatment slowed the progression of disability. Consequently, the status of interferon beta with respect to the rreatment of secondary progressive multiple sclerosis, with or without recent relapses, remains controversial. In a phase 3 European trial, mitoxantrone hydrochloride, an anthracenedione derivative and a cytotoxic agent with associated antiinflammatory activities, reduced both clinical and MRI evidence of disease activity in patients with secondary progressive multiple sclerosis.[97]

Primary Progressive Multiple Sclerosis and the Management of Symptoms

There are no proven therapies for primary progressive multiple sclerosis, although phase 3 trials of interferons and glatiramer acetate are under way. None of the treatments reverse the neurologic disabilities.

Treatment of Complications

There are moderately effective treatments for several of the complications of multiple sclerosis. Fatigue may respond to amantadine and to energy-conservation strategies. Depression and sleep disorders may contribute to fatigue and must be recognized and treated appropriately. Paroxysmal events typically respond well to carbamazepine and phenytoin (alone or in combination), acetazolamide, gabapentin, and pergolide.

Spasticity, pain, problems with gait, decubitus ulcers, speech and swallowing disorders, and cognitive and mood disorders are best treated by a multidisciplinary approach that may involve specialists in physical medicine and rehabilitation. Stretching, a program of aerobic exercise, and centrally acting muscle relaxants may help patients with mild, symptomatic spasticity. Patients with clinically significant weakness of the legs may require a moderate degree of extensor tone in order to walk and therefore may not be able to tolerate antispasticity medications. The implantation of a pump for the intrathecal administration of baclofen may assist in the management of intractable, painful spasticity in patients who cannot walk and who have lost bowel and bladder function. Neurogenic bladder and bowel disturbances are amenable to treatment after appropriate investigations have clarified the underlying physiologic mechanisms. Sexual dysfunction and chronic, central pain are common and may respond to appropriate symptom-based treatment strategies. Disabling, high-amplitude, cerebellar-outflow tremors rarely respond well to medication but may decrease after continued contralateral thalamic stimulation or ablative thalamotomy.

CHALLENGES IN CONDUCTING CLINICAL TRIALS AND FUTURE DIRECTIONS

Multiple sclerosis remains a challenging disease to study because the cause is unknown, the pathophysiologic mechanisms are diverse, and the chronic, unpredictable course of the disease makes it difficult to determine whether the favorable effects of short-term treatment will be sustained. Most published trials are small (usually including fewer than 150 patients per study group) and brief (less than three years of follow-up[98]). Clinical measures (the degree of disability, the relapse rate, and the time to clinical progression) remain the primary outcomes assessed in phase 3 trials. These measures are relatively insensitive to change and only weakly predictive of the long-term clinical outcome. No laboratory studies, including MRI, meet the requirements of the FDA for a surrogate marker of prognosis.

The important limitations of clinical trials involving patients with chronic illnesses, such as imperfect blinding, a high rate of withdrawal, and an incom-

pletely matched or inappropriate control group, are particularly prominent in studies of multiple sclerosis. In the past several years, trials have used increasingly sophisticated methods to identify promising agents as well as those that are toxic or ineffective.[99-101] Careful attention must be paid to the demographic characteristics of the control group before enrollment and to their clinical behavior after enrollment to avoid false positive results. For example, if the results in the control group are worse than those expected on the basis of the predicted natural history of the disease, the putative benefit of treatment in the other group may be exaggerated.

There is interest in designing trials to assess ways of delaying irreversible axonal injury and promoting remyelination. One strategy would be to evaluate whether combinations of drugs with different mechanisms of action are more effective than single-agent therapy. Other immunomodulating approaches include anticytokine and "immune-deviation" strategies, which are designed to favor the proliferation of antiinflammatory Th2 cells and Th2 cytokines (Fig. 2). Inhibitors of matrix metalloproteinases and other proteases, inhibitors of cathepsin B, inhibitors and scavengers of oxygen radicals, and efforts to reverse or reduce the activation of the trimolecular complex (including peptide immunotherapy and T-cell vaccination) may be worth additional study. Investigators who favor an infectious cause of multiple sclerosis, such as human herpesvirus type 6[60] or *C. pneumoniae*,[61] may initiate trials of antiviral and antibacterial agents. Other approaches focusing on reparative and remyelinative strategies include efforts to block antibody-mediated demyelination. It may be possible to enhance remyelination by transplanting oligodendroglial precursor cells into discrete, clinically important lesions (e.g., those affecting the optic nerves, the middle cerebellar peduncle, or the spinal cord)[102,103] while administering growth factors and neuroprotective agents. Gene-therapy strategies may also ultimately be worthy of study.

The widespread use of the partially effective immunomodulatory agents has left few patients who have not received such agents and who would therefore make good candidates for enrollment in trials. It may no longer be ethical to evaluate new treatments for relapsing–remitting multiple sclerosis in a placebo-controlled study except in unusual circumstances, such as those involving patients who have declined standard therapies or who have had no response to them and those involving brief trials in patients with recently diagnosed multiple sclerosis. The costs of definitive trials have also escalated markedly. Phase 3 studies should include at least three years of follow-up to identify biologically meaningful effects of treatment.[98,104]

During the past decade, there has been moderate progress in reducing the inflammatory component of multiple sclerosis. Unfortunately, most patients continue to have relapses and progression of their symptoms. This finding has forced a reexamination of the hypothesis that the elimination of acute relapses and, by inference, inflammation would be curative. An alternative hypothesis is that clinical progression is independent of inflammation but depends on factors intrinsic to the pathologic substrate influencing demyelination and, in particular, injury to axons. If this hypothesis is confirmed, newer approaches directed toward interfering with demyelination and axonal injury will be necessary to prevent progression and restore function. Many degenerative neurologic diseases share mechanisms of injury (e.g., apoptosis, oxidative stress, loss of trophic support, and proteolysis). As the margins between the neurodegenerative diseases begin to blur, unifying concepts of nervous system injury will emerge, providing opportunities for the design of rational treatments.

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REFERENCES

1. Compston A, Ebers G, Lassmann H, McDonald I, Matthews B, Wekerle H. McAlpine's multiple sclerosis. 3rd ed. London: Churchill Livingstone, 1998.
2. Noseworthy J. Progress in determining the causes and treatment of multiple sclerosis. Nature 1999;399:Suppl:A40-A47.
3. Anderson DW, Ellenberg JH, Leventhal CM, Reingold SC, Rodriguez M, Silberberg DH. Revised estimate of the prevalence of multiple sclerosis in the United States. Ann Neurol 1992;31:333-6.
4. Weinshenker BG, Bass B, Rice GP, et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain 1989;112:133-46.
5. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227-31.
6. Miller DH, Grossman RI, Reingold SC, McFarland HF. The role of magnetic resonance techniques in understanding and managing multiple sclerosis. Brain 1998;121:3-24.
7. Gronseth GS, Ashman EJ. Practice parameter: the usefulness of evoked potentials in identifying clinically silent lesions in patients with suspected multiple sclerosis (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2000;54:1720-5.
8. O'Riordan JI, Thompson AJ, Kingsley DP, et al. The prognostic value of brain MRI in clinically isolated syndromes of the CNS: a 10-year follow-up. Brain 1998;121:495-503.
9. Cole SR, Beck RW, Moke PS, Kaufman DI, Tourtellotte WW. The predictive value of CSF oligoclonal banding for MS 5 years after optic neuritis. Neurology 1998;51:885-7.
10. Weinshenker BG, Rice GPA, Noseworthy JH, Carriere W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study. 4. Applications to planning and interpretation of clinical therapeutic trials. Brain 1991;114:1057-67.
11. Cottrell DA, Kremenchutzky M, Rice GPA, Hader W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study. 6. Applications to planning and interpretation of clinical therapeutic trials in primary progressive multiple sclerosis. Brain 1999;122:641-7.
12. Sadovnick AD, Eisen K, Ebers GC, Paty DW. Cause of death in patients attending multiple sclerosis clinics. Neurology 1991;41:1193-6.
13. Weinshenker BG, Rodriguez M. Epidemiology of multiple sclerosis. In: Gorelick PB, Alter M, eds. Handbook of neuroepidemiology. Vol. 29 of Neurological disease and therapy. New York: Marcel Dekker, 1994:533-67.

14. Kurtzke JF. Multiple sclerosis: changing times. Neuroepidemiology 1991;10:1-8.
15. Rosati G, Aiello I, Pirastru MI, et al. Epidemiology of multiple sclerosis in northwestern Sardinia: further evidence for higher frequency in Sardinians compared to other Italians. Neuroepidemiology 1996;15:10-9.
16. Bufill E, Blesa R, Galan I, Dean G. Prevalence of multiple sclerosis in the region of Osona, Catalonia, northern Spain. J Neurol Neurosurg Psychiatry 1995;58:577-81.
17. Wynn DR, Rodriguez M, O'Fallon WM, Kurland LT. A reappraisal of the epidemiology of multiple sclerosis in Olmsted County, Minnesota. Neurology 1990;40:780-6.
18. Svenningsson A, Runmarker B, Lycke J, Andersen O. Incidence of MS during two fifteen-year periods in the Gothenburg region of Sweden. Acta Neurol Scand 1990;82:161-8.
19. Cook SD, Cromarty JI, Tapp W, Poskanzer D, Walker JD, Dowling PC. Declining incidence of multiple sclerosis in the Orkney Islands. Neurology 1985;35:545-51.
20. Midgard R, Riise T, Nyland H. Epidemiologic trends in multiple sclerosis in More and Romsdal, Norway: a prevalence/incidence study in a stable population. Neurology 1991;41:887-92.
21. Kurtzke JF, Hyllested K. Multiple sclerosis in the Faroe Islands. III. An alternative assessment of the three epidemics. Acta Neurol Scand 1987; 76:317-39.
22. Alter M, Leibowitz U, Speer J. Risk of multiple sclerosis related to age at immigration to Israel. Arch Neurol 1966;15:234-7.
23. Dean G. Annual incidence, prevalence, and mortality of multiple sclerosis in white South-African-born and in white immigrants to South Africa. BMJ 1967;2:724-30.
24. Elian M, Nightingale S, Dean G. Multiple sclerosis among United Kingdom-born children of immigrants from the Indian subcontinent, Africa and the West Indies. J Neurol Neurosurg Psychiatry 1990;53:906-11.
25. Compston A. Risk factors for multiple sclerosis: race or place? J Neurol Neurosurg Psychiatry 1990;53:821-3.
26. Ebers GC, Sadovnick AD, Risch NJ, Canadian Collaborative Study Group. A genetic basis for familial aggregation in multiple sclerosis. Nature 1995;377:150-1.
27. Sadovnick AD, Armstrong H, Rice GP, et al. A population-based study of multiple sclerosis in twins: update. Ann Neurol 1993;33:281-5.
28. Sadovnick AD, Baird PA, Ward RH. Multiple sclerosis: updated risks for relatives. Am J Med Genet 1988;29:533-41.
29. Jersild C, Fog T, Hansen GS, Thomsen M, Svejgaard A, Dupont B. Histocompatibility determinants in multiple sclerosis, with special reference to clinical course. Lancet 1973;2:1221-5.
30. Marrosu MG, Murru MR, Costa G, Murru R, Muntoni F, Cucca F. DRB1-DQA1-DQB1 loci and multiple sclerosis predisposition in the Sardinian population. Hum Mol Genet 1998;7:1235-7.
31. Sawcer S, Jones HB, Feakes R, et al. A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. Nat Genet 1996;13:464-8.
32. Ebers GC, Kukay K, Bulman DE, et al. A full genome search in multiple sclerosis. Nat Genet 1996;13:472-6.
33. Haines JL, Ter-Minassian M, Bazyk A, et al. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatability complex. Nat Genet 1996;13:469-71.
34. Kuokkanen S, Gschwend M, Rioux JD, et al. Genomewide scan of multiple sclerosis in Finnish multiplex families. Am J Hum Genet 1997;61: 1379-87.
35. Haines JL, Terwedow HA, Burgess K, et al. Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. Hum Mol Genet 1998;7:1229-34.
36. Chataway J, Feakes R, Coraddu F, et al. The genetics of multiple sclerosis: principles, background and updated results of the United Kingdom systematic genome screen. Brain 1998;121:1869-87.
37. te Meerman GJ, Van der Meulen MA. Genomic sharing surrounding alleles identical by descent: effects of genetic drift and population growth. Genet Epidemiol 1997;14:1125-30.
38. Robertson NP, Compston DAS. Prognosis in multiple sclerosis: genetic factors. In: Siva A, Kesselring J, Thompson AJ, eds. Frontiers in multiple sclerosis. Vol. 2. London: Martin Dunitz, 1999:51-61.
39. Weinshenker BG, Santrach P, Bissonet AS, et al. Major histocompatibility complex class II alleles and the course and outcome of MS: a population-based study. Neurology 1998;51:742-7.
40. Schrijver HM, Crusius JB, Uitdehaag BM, et al. Association of interleukin-1beta and interleukin-1 receptor antagonist genes with disease severity in MS. Neurology 1999;52:595-9.
41. Myhr KM, Raknes G, Nyland H, Vedeler C. Immunoglobulin G Fc-receptor (Fc γ R) IIA and IIIB polymorphisms related to disability in MS. Neurology 1999;52:1771-6.
42. Evangelou N, Jackson M, Beeson D, Palace J. Association of the APOE epsilon4 allele with disease activity in multiple sclerosis. J Neurol Neurosurg Psychiatry 1999;67:203-5.
43. Hohlfeld R. Biotechnological agents for the immunotherapy of multiple sclerosis: principles, problems and perspectives. Brain 1997;120:865-916.
44. Lassmann H, Raine CS, Antel J, Prineas JW. Immunopathology of multiple sclerosis: report on an international meeting held at the Institute of Neurology of the University of Vienna. J Neuroimmunol 1998;86:213-7.
45. Wolswijk G. Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. J Neurosci 1998; 18:601-9.
46. Waxman SG. Demyelinating diseases — new pathological insights, new therapeutic targets. N Engl J Med 1998;338:323-5.
47. Rivera-Quinones C, McGavern D, Schmelzer JD, Hunter SF, Low PA, Rodriguez M. Absence of neurological deficits following extensive demyelination in a class I-deficient murine model of multiple sclerosis. Nat Med 1998;4:187-93.
48. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. N Engl J Med 1998; 338:278-85.
49. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. A quantitative analysis of oligodendrocytes in multiple sclerosis lesions: a study of 113 cases. Brain 1999;122:2279-95.
50. Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Brück W. Acute axonal injury in multiple sclerosis: correlation with demyelination and inflammation. Brain 2000;123:1174-83.
51. Schmidt S, Linington C, Zipp F, et al. Multiple sclerosis: comparison of the human T-cell response to S100β and myelin basic protein reveals parallels to rat experimental autoimmune panencephalitis. Brain 1997;120: 1437-45.
52. van Noort JM, van Sechel AC, Bajramovic JJ, et al. The small heat-shock protein αB-crystallin as candidate autoantigen in multiple sclerosis. Nature 1996;375:798-801.
53. Steinman L. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. Cell 1996;85:299-302.
54. Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H. Multiple sclerosis: lessons from neuropathology. Semin Neurol 1998;18:337-49.
55. Piddlesden SJ, Lassmann H, Zimprich F, Morgan BP, Linington C. The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement. Am J Pathol 1993; 143:555-64.
56. Linington C, Bradl M, Lassmann H, Brunner C, Vass K. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. Am J Pathol 1988;130:443-54.
57. Raine CS, Cannella B, Hauser SL, Genain CP. Demyelination in primate autoimmune encephalomyelitis and acute multiple sclerosis lesions: a case for antigen-specific antibody mediation. Ann Neurol 1999;46:144-60.
58. Storch MK, Piddlesden S, Haltia M, Iivanainen M, Morgan P, Lassmann H. Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. Ann Neurol 1998;43:465-71.
59. Carrigan DR, Harrington D, Knox KK. Subacute leukoencephalitis caused by CNS infection with human herpesvirus-6 manifesting as acute multiple sclerosis. Neurology 1996;47:145-8.
60. Challoner PB, Smith KT, Parker JD, et al. Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. Proc Natl Acad Sci U S A 1995;92:7440-4.
61. Sriram S, Stratton CW, Yao S, et al. Chlamydia pneumoniae infection of the central nervous system in multiple sclerosis. Ann Neurol 1999;46: 6-14.
62. Boman J, Roblin PM, Sundstrom P, Sandstrom M, Hammerschlag MR. Failure to detect Chlamydia pneumoniae in the central nervous system of patients with MS. Neurology 2000;54:265.
63. Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H. Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. Brain Pathol 1996;6:259-74.
64. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000;47:707-17.
65. Paty DW, Li DK, UBC MS/MRI Study Group, IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993;43:662-7.
66. The IFNB Multiple Sclerosis Study Group, University of British Columbia MS/MRI Analysis Group. Interferon β-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. Neurology 1995;45:1277-85.
67. Simon JH, Jacobs LD, Campion M, et al. Magnetic resonance studies

of intramuscular interferon β-1a for relapsing multiple sclerosis. Neurology 1998;43:79-87.
68. PRISMS (Prevention of Relapses and Disability by Interferon β-1a Subcutaneously in Multiple Sclerosis) Study Group. Randomised double-blind placebo-controlled study of interferon β-1a in relapsing/remitting multiple sclerosis. Lancet 1998;352:1498-504. [Erratum, Lancet 1999; 353:678.]
69. Miller DH, Molyneux PD, Barker GJ, MacManus DG, Moseley IF, Wagner K. Effect of interferon β-1b on magnetic resonance imaging outcomes in secondary progressive multiple sclerosis: results of a European multicenter, randomized, double-blind, placebo-controlled trial. Ann Neurol 1999;46:850-9.
70. Filippi M, Rocca MA, Martino G, Horsfield MA, Comi G. Magnetization transfer changes in the normal appearing white matter precede the appearance of enhancing lesions in patients with multiple sclerosis. Ann Neurol 1998;43:809-14.
71. Allen IV, McKeown SR. A histological, histochemical and biochemical study of the macroscopically normal white matter in multiple sclerosis. J Neurol Sci 1979;41:81-91.
72. Goodkin DE, Rooney WD, Sloan R, et al. A serial study of new MS lesions and the white matter from which they arise. Neurology 1998;51: 1689-97.
73. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T, Pregnancy in Multiple Sclerosis Group. Rate of pregnancy-related relapse in multiple sclerosis. N Engl J Med 1998;339:285-91.
74. Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. Lancet 1985;1:1313-5.
75. Miller AE, Morgante LA, Buchwald LY, et al. A multicenter, randomized, double-blind, placebo-controlled trial of influenza immunization in multiple sclerosis. Neurology 1997;48:312-4.
76. European Study Group on Interferon β-1b in Secondary Progressive MS. Placebo-controlled multicentre randomised trial of interferon β-1b in treatment of secondary progressive multiple sclerosis. Lancet 1998;352: 1491-7.
77. Goodkin DE, North American Study Group on Interferon beta-1b in Secondary Prevention MS. Interferon beta-1b in secondary progressive MS: clinical and MRI results of a 3-year randomized controlled trial. Neurology 2000;54:Suppl:2352. abstract.
78. Tubridy N, Behan PO, Capildeo R, et al. The effect of anti-alpha4 integrin antibody on brain lesion activity in multiple sclerosis. Neurology 1999;53:466-72.
79. Beck RW, Cleary PA, Anderson MM Jr, et al. A randomized, controlled trial of corticosteroids in the treatment of acute optic neuritis. N Engl J Med 1992;326:581-8.
80. Beck RW. The Optic Neuritis Treatment Trial: three-year follow-up results. Arch Ophthalmol 1995;113:136-7.
81. Weinshenker BG, O'Brien PC, Petterson TM, et al. A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease. Ann Neurol 1999;46:878-86.
82. Comi G, Filippi M, Barkhof F, et al. Interferon beta 1a (Rebif) in patients with acute neurological syndromes suggestive of multiple sclerosis: a multi-center, randomized, double-blind, placebo-controlled study. Neurology 2000;54:Suppl 3:A85-A86. abstract.
83. Jacobs LD, Beck RW, Simon JH, et al. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. N Engl J Med 2000;343:898-904.
84. The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993;43:655-61.
85. Johnson KP, Brooks BR, Cohen JA, et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. Neurology 1995;45:1268-76.
86. Johnson KP, Brooks BR, Cohen JA, et al. Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. Neurology 1998;50:701-8.
87. Jacobs LD, Cookfair DL, Rudick RA, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. Ann Neurol 1996;39:285-94. [Erratum, Ann Neurol 1996;40:480.]
88. Rudick RA, Goodkin DE, Jacobs LD, et al. Impact of interferon beta-1a on neurologic disability in relapsing multiple sclerosis. Neurology 1997; 49:358-63.
89. Forbes RB, Lees A, Waugh N, Swingler RJ. Population based cosr urility study of interferon beta-1b in secondary progressive multiple sclerosis. BMJ 1999;319:1529-33.
90. van den Noort S, Eidelman B, Rammohan K, et al. National Multiple Sclerosis Society (NMSS): disease management consensus statement. New York: National MS Society, 1998.
91. Rodriguez M, Siva A, Ward J, Stolp-Smith K, O'Brien P, Kurland L. Impairment, disability, and handicap in multiple sclerosis: a population-based study in Olmsted County, Minnesota. Neurology 1994;44:28-33.
92. The Once Weekly Interferon for MS Study Group. Evidence of interferon beta-1a dose response in relapsing-remitting MS. Neurology 1999; 53:679-86.
93. Freedman MS, PRISMS Study Group. PRISMS 4-year results: evidence of clinical dose effect of interferon beta-1a in relapsing MS. Neurology 2000;54:Suppl:2351. abstract.
94. Fazekas F, Deisenhammer F, Strasser-Fuchs S, Nahler G, Mamoli B. Randomised placebo-controlled trial of monthly intravenous immunoglobulin therapy in relapsing-remitting multiple sclerosis. Lancet 1997;349: 589-93.
95. Paty D. Results of the 3-year, double-blind, placebo-controlled study of interferon beta-1a (Rebif) in secondary-progressive MS. Presented at the Ninth Annual Meeting of the European Neurological Society, Milan, Italy, June 5–9, 1999.
96. Hughes R, SPECTRIMS Group. "Relapsing" versus "nonrelapsing" SPMS: different prognosis and response to interferon therapy in the SPECTRIMS study. Neurology 2000;54:Suppl 3:A233. abstract.
97. Krapf H, Morrissey SP, Zenker O, et al. Mitoxantrone in progressive multiple sclerosis: MRI results of the European phase III trial. Neurology 1999;52:Suppl 2:A495. abstract.
98. Rudge P. Are clinical trials of therapeutic agents for MS long enough? Lancet 1999;353:1033-4.
99. Noseworthy JH, Gold R, Hartung H-P. Treatment of multiple sclerosis: recent trials and future perspectives. Curr Opin Neurol 1999;12:279-93.
100. van Oosten BW, Barkhof F, Truyen L, et al. Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2. Neurology 1996;47: 1531-4.
101. The Lenercept Multiple Sclerosis Study Group, University of British Columbia MS/MRI Analysis Group. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. Neurology 1999;53: 457-65.
102. Compston A. Remyelination in multiple sclerosis: a challenge for therapy: the 1996 European Charcot Foundation Lecture. Mult Scler 1997;3:51-70.
103. Archer DR, Cuddon PA, Lipsitz D, Duncan LD. Myelination of the canine central nervous system by glial cell transplantation: a model for repair of human myelin disease. Nat Med 1997;3:54-9.
104. Noseworthy JH, O'Brien P, Erickson BJ, et al. The Mayo Clinic-Canadian Cooperative Trial of sulfasalazine in active multiple sclerosis. Neurology 1998;51:1342-52.

SPECIAL ARTICLE

New Diagnostic Criteria for Multiple Sclerosis: Guidelines for Research Protocols

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Several schemes for the diagnosis and clinical classification of multiple sclerosis (MS) have been advanced [1]. The best known is that published by Schumacher et al [3]. The criteria for this scheme were established in order to select patients for participation in therapeutic trials, and pertain only to what might be called definite MS. No provision was made for incorporating supportive laboratory data into the diagnostic criteria.

As no reliable specific laboratory test for the diagnosis of MS has been discovered, the diagnosis remains a clinical one, and there is still a need for clinical diagnostic criteria. However, several laboratory and clinical procedures have been developed within the last decade which aid greatly in demonstrating neurological dysfunction attributable to lesions, and even the lesions themselves.

One problem with the various published diagnostic classifications is their discrepant terminology: what is considered "probable" in one is called "definite" in another. Another problem is that all the proposed schemes require much subjective judgment, a difficulty which cannot be completely overcome but can be diminished by adding to the clinical evaluation the results of laboratory, neuroimaging, neuropsychological, and neurophysiological procedures. Today there is a need for more exact criteria than existed earlier in order to conduct therapeutic trials in multicenter programs, to compare epidemiological surveys, to evaluate new diagnostic procedures, and to estimate the activity of the disease process in MS.

Method and Procedure

On April 26 and 27, 1982, the following persons participated in a Workshop on the Diagnosis of Multiple Sclerosis, held in Washington, DC, for the purpose of establishing new diagnostic criteria for MS: Bruce Becker (National Naval Medical Center), Jerry Blaivas (Columbia), Keith Chiappa (Harvard), Floyd Davis (Rush), Burton Drayer (Duke), George Ebers (Western Ontario), Andrew Eisen (British Columbia), Robert Herndon (Rochester, NY), Kenneth Johnson (Maryland), Ian McDonald (National Hospital, London), Dale McFarlin (NINCDS), Donald Paty, Co-chairman (British Columbia), Janis Peyser (Vermont), Charles Poser, Chairman (Boston), David Regan (Dalhousie), Daniel Sax (Boston), Labe Scheinberg, Co-chairman (Albert Einstein), Simon Sears (Texas-Houston), William Sibley (Arizona), Donald Silberberg (Pennsylvania), Robert Slater (National MS Society), Emanuel Stadlan (NINCDS), Wallace Tourtellotte (Wadsworth VA/UCLA), and Byron Waksman (National MS Society). Dr Robert Daroff (Case–Western Reserve) made many useful suggestions. The disciplines represented included neurology, neuropsychology, urology, immunology, neuroradiology, neuroophthalmology, clinical neurophysiology, and neuropathology.

The participants reviewed in detail historical and clinical symptomatology in MS; immunological observations; cerebrospinal fluid (CSF) tests; neurophysiological procedures including visual, brainstem auditory, trigeminal, and somatosensory evoked potential measurements; the evoked blink reflex; a variety of physiological and psychophysiological procedures; neuropsychological assessment; tissue imaging procedures such as computer assisted tomography (CT scanning) and nuclear magnetic resonance (NMR); and urological studies of bladder, bowel, and sexual dysfunction. This re-

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view resulted in formulation of guidelines for the performance of these procedures and for evaluation of the results that will be published with recommendations regarding their usefulness in the diagnosis of MS [2]. The diagnostic criteria presented here represent the views of the majority of the workshop participants.

Definitions

1. *Attack (bout, episode, exacerbation):* The occurrence of a symptom or symptoms of neurological dysfunction, with or without objective confirmation, lasting more than 24 hours constitutes an attack. This may be completely subjective and anamnestic, e.g., the patient reports having had double vision for three days but did not consult a physician; or numbness and tingling of a leg caused a visit to a physician who was unable to demonstrate objective changes; or the patient was hospitalized because of severe ataxia and was found to have signs of cerebellar dysfunction, bilateral Babinski signs, and left facial weakness. Individual symptoms, however, may last for considerably less time than that: e.g., a Lhermitte sign (which is really a symptom) or vertigo may last for only seconds; these manifestations cannot be considered attacks in this context.

2. *Historical information:* The description of symptoms by the patient. The example just cited (under the definition of attack) of the episode of diplopia would be historical, and so would the leg numbness, although medical corroboration would strengthen the latter. Ideally, medical records which confirm anamnestic information should be obtained.

3. *Clinical evidence of a lesion: Signs* of neurological dysfunction demonstrable by neurological examination. Such neurological signs are acceptable even if no longer present, provided that they were elicited and recorded in the past by a competent examiner.

4. *Paraclinical* evidence of a lesion:* The demonstration by means of various tests and procedures of the existence of a lesion of the central nervous system (CNS) which has not produced *signs* of neurological dysfunction but which may or may not have caused *symptoms* in the past. Such tests and procedures include the hot bath test, evoked response studies, tissue imaging procedures, and reliable, expert urological assessment, provided that these tests and procedures follow the guidelines and are interpreted according to the newly established criteria to be published [2]. These diagnostic procedures represent various options, all of which may not be available and some of which may not be deemed suitable or reliable enough by individual neurologists.

**Webster's Third New International Dictionary,* unabridged, 1971, gives the following definitions for para-: 1a. beside, alongside of; 1d. associated in a subsidiary or accessory capacity. *Paraclinical* would appear more suitable than *subclinical.*

5. *Typical of MS:* MS is known to involve certain parts of the CNS much more frequently than others, and thus certain signs and symptoms are more frequently noted. Gray matter lesions occur rarely enough in MS that they should not be considered in establishing the diagnosis. Lesions of the peripheral nervous system, except when accounted for by their intramedullary course (e.g., oculomotor, trigeminal, or facial nerves), may not be counted. Complaints such as headaches, convulsive seizures, depression, or alterations of the state of consciousness are too nonspecific to be considered in the diagnostic construct.

6. *Remission:* A definite improvement of signs, symptoms, or both that has been present for at least 24 hours is called a remission for the purpose of these guidelines. A remission must last at least one month to be considered significant.

7. *Separate lesions:* Separate signs or symptoms cannot be explainable on the basis of a single lesion; simultaneously occurring internuclear ophthalmoplegia, facial weakness, and signs of involvement of the corticospinal tracts could have been caused by a single lesion (e.g., brainstem infarction) and thus would not be acceptable. Optic neuritis involving both eyes occurring simultaneously, or the second eye becoming involved within 15 days of the first (provided that compression of the chiasm by tumor or aneurysm has been ruled out), is considered to represent a single lesion. Only lesions that involve distinctly different parts of the CNS are called separate lesions.

8. *Laboratory support:* The term is applied here only to the examination of CSF for oligoclonal bands and increased production of immunoglobulin G (IgG). All other laboratory procedures, such as evoked responses or tissue imaging techniques, are considered to be extensions of the clinical examination.

General Considerations

The acceptable age of onset for research purposes is between 10 and 59 years inclusive. The manifestations of the disease offered in evidence must be shown to be characteristic of MS and not attributable to another condition. Such a decision must be made by a physician who is experienced in clinical neurology. It is strongly recommended that the diagnosis of MS be established only by a competent neurologist. Although extended and expensive investigations are not encouraged, other illnesses capable of producing signs and symptoms of multiple lesions of the CNS must be considered. More important, clinical observation over several weeks or months may obviate the need for much laboratory investigation. A steadily progressive disease from onset, without reliable evidence of exacerbations or remissions, with manifestations reflecting a single lesion, and without paraclinical evidence of a lesion elsewhere in the CNS is not to be classified as MS for research

Category	Attacks	Clinical Evidence		Paraclinical Evidence	CSF OB/IgG
A. Clinically definite					
CDMS A1	2	2			
CDMS A2	2	1	and	1	
B. Laboratory-supported definite					
LSDMS B1	2	1	or	1	+
LSDMS B2	1	2			+
LSDMS B3	1	1	and	1	+
C. Clinically probable					
CPMS C1	2	1			
CPMS C2	1	2			
CPMS C3	1	1	and	1	
D. Laboratory-supported probable					
LSPMS D1	2				+

OB/IgG = oligoclonal bands or increased IgG.

purposes, even in the presence of oligoclonal bands or increased IgG production in the CSF. Most neurological clinicians will regard such patients as probable cases of MS; nevertheless, they should not be enrolled in research protocols.

Classification of Multiple Sclerosis

The proposed classification of MS for use in research protocols consists of two major groups, definite and probable, each with two subgroups, clinical and laboratory supported (Table). The traditional possible MS group is not included because patients so labeled would not be acceptable for research studies.

A. *Clinically definite MS (CDMS)*
   1. Two attacks and clinical evidence of two separate lesions
   2. Two attacks; clinical evidence of one lesion and paraclinical evidence of another, separate lesion

COMMENT. The two attacks must involve different parts of the CNS, must be separated by a period of at least one month, and must each last a minimum of 24 hours.

Certain historical information may be substituted for clinical evidence of one of the two lesions (in category A1) if it fulfills the following conditions: the information is reliable, is adequate to localize a lesion typical of MS, and has no other explanation. Examples include a Lhermitte sign in any person under the age of 50 years who does not have radiologically demonstrable evidence of cervical spine disease; a useless hand due to severe impairment of position sense causing severe stereoanesthesia; a typical optic neuritis occurring before the age of 50 with loss of vision and with pain on motion of the eye or, if no substantial loss of vision has occurred, with description of visual field defect or alteration of color vision; transient paraparesis with paresthesias; oscillopsia; typical diplopia (in the absence of thyroid disease or a prior history of orbital trauma) that is abolished by closing either eye; and trigeminal neuralgia with onset before the age of 40. Extreme caution must be exercised in making such a substitution. If possible, confirmation by a relative or friend should be obtained if the attack was not observed and recorded by a physician.

Many individuals have become quite familiar with the symptoms of MS from articles published in lay magazines and other easily available sources of information. MS Munchausens are known to exist, and establishment of the diagnosis of MS may be of advantage to some individuals in some circumstances.

Paraclinical evidence of CNS lesions may be elicited by a variety of means, including induced hyperthermia, evoked potential studies, CT and NMR scans, or special urological studies. Neuropsychological evaluation by an expert examiner that indicates definite cognitive impairment in a patient under the age of 50 may be suggestive and helpful but not yet specific enough to be fully diagnostic. No other explanation for these lesions must be evident. Use of the procedures and evaluation of results must follow the guidelines, which will be published shortly [2].

B. *Laboratory-supported definite MS (LSDMS)*
   The laboratory support consists of demonstration in CSF of IgG oligoclonal bands (OB) or of increased CNS synthesis of IgG. Oligoclonal bands must not

be present in the patient's serum, and the serum IgG level must be normal. This assumes that other conditions causing CSF changes, such as syphilis, subacute sclerosing panencephalitis, sarcoidosis, collagen vascular disease, and similar disorders, have been ruled out.

1. Two attacks; either clinical or paraclinical evidence of one lesion; and CSF OB/IgG

COMMENT. The two attacks must involve different parts of the CNS and be separated by a minimum of one month, each having lasted at least 24 hours. One of the episodes must involve a part of the CNS distinct from that demonstrated by the clinical or paraclinical evidence.

2. One attack; clinical evidence of two separate lesions; and CSF OB/IgG
3. One attack; clinical evidence of one lesion and paraclinical evidence of another, separate lesion; and CSF OB/IgG

COMMENT. Historical information cannot be substituted for the clinical evidence. Whether the evidence is clinical or paraclinical, both lesions must not have been present at the time of the first examination and must be separated by at least one month. This separation in time is designed to reduce the possibility of including a case of acute disseminated encephalomyelitis. In a patient with the so-called progressive form of MS, i.e., without remissions and exacerbations, evidence of clinical or paraclinical optic nerve involvement, for example, should not have been present at the time the paraparesis first appeared. Under those circumstances, and only if steady progression has taken place for at least six months, may such a case be accepted as MS.

C. *Clinically probable MS (CPMS)*
1. Two attacks and clinical evidence of one lesion

COMMENT. The two attacks must involve separate parts of the CNS. Historical information cannot be considered as a substitute for the clinical evidence.

2. One attack and clinical evidence of two separate lesions
3. One attack; clinical evidence of one lesion and paraclinical evidence of another, separate lesion

COMMENT. See under B3.

D. *Laboratory-supported probable MS (LSPMS)*
1. Two attacks and CSF OB/IgG

COMMENT. The two attacks must involve different parts of the CNS, must be separated by a minimum of one month, and must each have lasted at least 24 hours.

Discussion

The main reason for establishing these criteria is to restrict therapeutic trials and other research protocols to patients with definite MS; the category of probable is designed for the purpose of prospectively evaluating new diagnostic methods. The introduction of the categories of laboratory-supported definite and probable MS extends the limits of the diagnostic criteria, thus making available a larger reservoir of patients for investigative purposes. Naturally, investigators retain the prerogative of availing themselves of this additional group of patients or restricting their choice on the basis of the classic clinical criteria.

The guidelines may appear unduly complicated to the neurological practitioner. They are not meant to deter the clinician in the effort to establish a diagnosis of MS. They will not replace the intuitive feelings derived from subtle indices that so often lead an experienced physician to the solution of the problem; rather, they should help guide the diagnostic investigation in the right direction. To a physician, the distinction between definite and probable MS may matter very little. To a patient, the end of uncertainty is important. If the guidelines result in diminution of the patient's (and the family's) search for alternative or confirmatory opinions, they will be worthwhile.

A major concern in establishing diagnostic criteria for MS is differentiation of the disease from acute disseminated encephalomyelitis (ADEM) with its multiple separate lesions. With rare exceptions, ADEM is a monophasic illness, all its lesions occurring within a couple of weeks in most instances. Patients with ADEM may also have CSF oligoclonal bands or increased CNS production of IgG. The problem of steadily progressive myelopathy is equally difficult to resolve, and a prolonged period of observation may be necessary. The need to make the diagnostic criteria fairly rigid for the intended purposes means that some types of patients will not fit any of the proposed categories despite the fact that many neurologists would consider them to have definite MS; for example, a young woman who during the course of an employment physical examination is found to have monocular optic atrophy, sustained nystagmus on left lateral gaze, and a right Babinski sign but who denies ever having had symptoms referable to the CNS will almost certainly be so diagnosed, as will a young man who, following an automobile accident, is found to have several separate, contrast-enhancing periventricular lesions on CT scan. The former patient in fact may well have had a single episode of ADEM that manifested itself only as a cou-

ple of days of headache, malaise, and slight nausea, a constellation of symptoms hardly suggestive of MS. It can be argued that such asymptomatic patients should not be included as subjects for therapeutic trials.

The Schumacher criteria have served us well, but presently available reliable and productive ancillary procedures must be incorporated into more up-to-date guidelines. These diagnostic criteria were developed to delineate groups of patients whose diagnosis will be accepted by a wide range of investigators worldwide for inclusion in various studies and protocols.

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References

1. Brown JR, Beebe GW, Kurtzke JF, Loewenson RB, Silberberg DH, Tourtellotte WW: The design of clinical studies to assess therapeutic efficacy in multiple sclerosis. Neurology (NY) 29(2):3–23, 1979
2. Poser CM, Paty DW, Scheinberg L, McDonald WI, Ebers GC (eds): The Diagnosis of Multiple Sclerosis. New York, Thieme-Stratton (in press)
3. Schumacher GA, Beebe GW, Kibler RF, Kurland LT, Kurtzke JF, McDowell F, Nagler B, Sibley WA, Tourtellotte WW, Willmon TL: Problems of experimental trials of therapy in multiple sclerosis. Ann NY Acad Sci 122:552–568, 1965

(12)

Cladribine and progressive MS

Clinical and MRI outcomes of a multicenter controlled trial

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Article abstract—*Objective:* To evaluate the safety and efficacy of two doses of cladribine in patients with progressive MS. *Background:* Treatment of progressive MS patients with cladribine in a previous single-center, placebo-controlled clinical trial was associated with disease stabilization. *Methods:* In the current study, 159 patients with a median baseline Kurtzke's Expanded Disability Status Scale (EDSS) score of 6.0 were randomly assigned to receive placebo or cladribine (0.07 mg/kg/day for 5 consecutive days every 4 weeks for either two or six cycles (total dose, 0.7 mg/kg or 2.1 mg/kg, respectively), followed by placebo, for a total of eight cycles. Thirty percent had primary progressive MS (PPMS) and 70% had secondary progressive MS (SPMS). EDSS and Scripps Neurologic Rating Scale (SNRS) scores were assessed bimonthly and MRI was performed every 6 months. The primary outcome measure was disability (mean change in EDSS). *Results:* Mean changes in disability did not differ among the groups at the end of the 12-month double-blind phase. Both cladribine treatments were superior to placebo for the proportion of patients having gadolinium-enhanced T1 lesions and for the mean volume and number of such lesions ($p \leq 0.003$). Differences were statistically significant at the 6-month evaluation time, with ≥90% reduction in volume and number of enhanced T1 lesions, which was maintained through final evaluation. This effect segregated largely with the SPMS group. The T2 burden of disease showed a modest improvement in cladribine-treated patients and worsened in placebo-treated patients. Most adverse events were mild or moderate in severity and not treatment limiting. *Conclusion:* No significant treatment effects were found for cladribine in terms of changes in EDSS or SNRS scores. Both doses of cladribine produced and sustained significant reductions in the presence, number, and volume of gadolinium-enhanced T1 brain lesions on MRI, and cladribine 2.1 mg/kg reduced the accumulation of T2 lesion load. Cladribine at doses up to 2.1 mg/kg was generally safe and well tolerated. **Key words:** Cladribine—MRI—Progressive MS—Suppression of disease activity.

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With the exception of trauma, MS—a demyelinating disease of the CNS with an estimated prevalence of 250,000 to 350,000 in the United States and 1.1 million worldwide—is the most common cause of neurologic disability in young adults.[1] About two thirds of patients develop a relapsing-remitting pattern (RRMS), and the majority of these will experience a progressive deterioration, or secondary progressive MS (SPMS); about 15% of patients appear to have a progressive course from onset, or primary progressive MS (PPMS).[2] The mandate for prevention of disease progression is compelling. The natural history of progressive MS has been little altered, at least in the short term, by currently available agents. β-Interferons have been reported to be effective in the treatment of RRMS,[3-13] and recently, interferon β-1b has been reported to delay the time to confirmed progression in patients with SPMS by 9 to 12 months.[14]

MRI has allowed direct visualization of the number, location, and volume of acute and chronic lesions associated with underlying disease pathology, and some correlations between MRI and clinical parameters have been demonstrated.[15] In patients with RRMS and SPMS, there is a correlation between the frequency and extent of lesion enhancement and short-term disease activity.[16-19] In clinical trials, the presence of contrast-enhanced T1 lesions at baseline has been shown to predict both clinical and MRI activity in the following 6 months,[19] and, in patients with clinically isolated syndromes suggestive of MS, T2 lesion load at presentation is strongly correlated with disability after 5 years.[20,21] A recent meta-analysis of data from nine studies in 307 patients with RRMS and SPMS, however, found that although enhancement predicts the occurrence of relapses it is not a strong predictor of subsequent accumulation of disability over a 2-year period of observation.[22] Phase III clinical trials evaluating new therapies for MS now almost always include MRI evaluations along with traditional clinical assessments.[15,23]

Cladribine (2-chlorodeoxyadenosine; 2-CdA) is a purine nucleoside analogue resistant to the action of

*See Appendix 1 on page 1154 for a listing of members of the Cladribine Study Group and the Cladribine MRI Study Group.

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adenosine deaminase, which results in preferential lymphocytotoxicity. In cells with a high ratio of deoxycytidine kinase to deoxynucleotidase (e.g., lymphocytes and monocytes), cladribine is phosphorylated into the active triphosphate deoxynucleotide, 2-CdATP, which accumulates, causing a disruption of cellular metabolism, DNA damage, and subsequent cell death.[24] Its long-lasting lymphocytotoxic activity suggests that cladribine could be useful in modulating autoimmune processes involving lymphocyte abnormalities such as MS. Sipe and colleagues have reported the outcome of a placebo-controlled clinical trial of cladribine in patients with progressive MS.[25,26] Treatment with a total dose of 2.8 mg/kg cladribine was associated with significant stabilization of the disease in patients with SPMS. Compared with a progression rate of 50% of the patients treated with placebo, 95% of cladribine-treated patients were stable at 1 year. These clinical observations were supported by favorable effects in the MRI brain scans, i.e., nearly complete elimination of enhanced T1 lesions and stabilization of T2 lesion volume at final evaluation. Encouraged by this single-center study, a multicenter, double-blind, placebo-controlled trial was conducted to evaluate the safety and efficacy of two doses of cladribine in patients with progressive MS.

Methods. *Study population.* A total of 159 patients with progressive MS were enrolled at six clinical centers in the United States and Canada. Inclusion criteria for entry into the trial were clinically definite or laboratory-supported MS according to the Schumacher criteria[27] or Poser criteria[28] and defined as chronic progressive by the slow progression of signs and symptoms over the preceding 12 months; a baseline Expanded Disability Status Scale (EDSS)[29] score between 3.0 and 6.5; age 21 to 60 years; serum creatinine levels <1.5 mg/dL and creatinine clearance ≥ 80% of age-adjusted normal value; aspartate and alanine transaminase (AST and ALT) and alkaline phosphatase levels less than twice the normal upper limit; neutrophil count >1600/μL and platelet count >130,000/μL; and clinically normal ECG and chest x-ray. Patients were excluded from the trial if there was significant history of medical disease within the preceding 2 years that would impair participation in the trial; use of corticosteroids or other immunosuppressants such as cyclophosphamide, azathioprine, cyclosporine, or β-interferon within the preceding 3 months; total lymphoid irradiation; persistent leukopenia or thrombocytopenia after treatment with immunosuppressive agents; history of alcohol or drug abuse within the preceding year or of attempted suicide; malignancy or history of malignancy within the preceding 5 years; pregnancy or nursing; positive test result for HIV; use of an experimental drug or device within the preceding 60 days; or prior participation in a trial with cladribine. The protocol was approved by the respective institutional review boards, and patients signed informed consent forms.

Study design. This multicenter trial was a randomized, double-blind, parallel-group, placebo-controlled study designed to compare the safety and efficacy of two doses of cladribine and placebo administered by subcutaneous (SC) injection in patients with progressive MS, to evaluate the dose-response relationship, and to obtain information concerning the duration of any effects. The study included a 4-week screening phase, a 1-year double-blind phase, and a 6-year long-term extension. Patients were assigned to one of three parallel treatment groups (cladribine, 2.1 mg/kg; cladribine, 0.7 mg/kg; or placebo) according to a computer-generated randomization schedule stratified by baseline disease severity and site. Sample size computation was based on an assumed SD of 1.7 for change from the baseline EDSS score. The planned sample size of 50 patients per treatment group would have a statistical power of 80% based on a two-sided alpha of 0.05 to detect a difference of 1.0 in change from the baseline EDSS score between the cladribine, 2.1 mg/kg, and placebo groups.

The trial was initiated in December 1994. During the 1-year double-blind phase, patients were evaluated monthly for vital signs, adverse events, and a complete blood count (CBC) that was obtained just before the monthly visit. Neurologic status was evaluated bimonthly by assessment of EDSS and Scripps Neurologic Rating Scale (SNRS) scores by the blinded clinical investigators, who underwent standardized training. Brain MRI scans were obtained at baseline and months 6 and 12, as were total lymphocyte count and lymphocyte subset counts ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+$ plus $CD56^+$, and $CD4^+/CD8^+$ ratio). Physical examinations were performed at baseline and months 4, 8, and 12; a chemistry panel and urinalysis were performed periodically, and an ECG was obtained at the end of the treatment phase. During the first year of the post-double-blind follow-up phase, EDSS scores, CBC, and lymphocyte counts were assessed quarterly; MRI scans were obtained at months 18 and 24.

In addition to the treating physician, an examining physician was designated at each site to assess the patient's neurologic function using EDSS and SNRS scoring. All study investigators and patients were blinded to treatment assignment; adverse events and unblinded hematology results were routinely reviewed by an independent safety monitoring board. After all patients at a study site completed the 12-month double-blind phase, the blind was broken, and patients who fulfilled the hematologic dosing criteria were permitted to receive open-label cladribine treatment during the long-term extension phase of the study, provided at least 12 months had elapsed since the last dose of cladribine and there was evidence of disease progression. Patients treated with open-label cladribine were evaluated monthly for 12 months following initiation of the drug, and then quarterly.

Study medications and dosage. Patients who met the protocol-specified entry criteria were randomized in approximately equal numbers to receive eight monthly courses of therapy. Patients received six courses of cladribine 0.07 mg/kg/day SC for 5 consecutive days (total dose, 2.1 mg/kg), followed by two courses of placebo or two courses of cladribine 0.07 mg/kg/day SC for 5 consecutive days (total dose, 0.7 mg/kg), followed by six courses of placebo or eight courses of placebo SC for 5 consecutive days. To receive a subsequent course of blinded study drug, patients were required to meet the hematologic criteria, which were based on the results of a CBC obtained 2 to 4 days before each dosing period and are listed in Appen-

dix 2. For a patient who did not meet these criteria, placebo was substituted for the active drug for that dosing period. If the hematologic criteria for dosing were met at the next evaluation, the patient received active drug the following month, up to the eighth month. All CBC data were reviewed by an independent third party. The treating physician remained blinded but was provided with any abnormal CBC results required for proper medical management.

Concomitant therapy. Methylprednisolone, 1 g/day for up to 5 days, was allowed only for treatment of severe exacerbations. In addition, patients were allowed to continue receiving symptomatic therapies to treat troublesome symptoms of MS (e.g., baclofen for spasticity or oxybutynin chloride for bladder dysfunction).

MRI evaluation. Dual-echo conventional spin-echo images were obtained using repetition times of 2500 msec and echo times of 30 (proton-density weighting) and 90 (T2 weighting) msec. T1-weighted images were obtained using repetition times of 600 msec and echo times of 20 msec. For both sequences, slices were axial with a matrix size of 256 × 256 mm and a field of view of 200 × 200 mm. Sections were 4 mm thick with a 1-mm interslice gap for the dual-echo scans and 3 mm thick and contiguous for the T1-weighted scans. The total imaging time was approximately 20 to 25 minutes. Special attention was given to careful repositioning of the patient, using laser guidance and external landmarks to help achieve reproducible slice positions. All scan data were blinded to treatment, date, and sequence of scan.

Lesion identification. *Postcontrast T1-weighted images.* A single experienced observer identified enhanced lesions following rules and criteria established in recently published guidelines.[30] Areas of enhancement were marked on transparent sheets superimposed over the scan hard copies, and then the total number of enhanced lesions per scan was counted. Corresponding dual-echo images were used to increase the confidence in lesion detection.

T2-weighted images. A single experienced observer identified hyperintense MS lesions and marked the corresponding areas on transparent sheets superimposed over the proton-density scan hard copies. Corresponding T2-weighted images were used to increase the confidence in lesion detection.

Lesion segmentation and measurement of lesion volume. Trained technicians measured the lesion volumes for the scans belonging to the same patient to avoid variabilities of interobserver measurement. A local thresholding technique was used for lesion segmentation on computer-displayed images, with the marked hard copies kept as a reference. This local thresholding technique for segmentation was provided by the Dispunc display software for MR images, developed by David Plummer (University College, London, UK). The observer first chooses a point on the lesion using a mouse-controlled cursor, and the algorithm starts contouring, following from the strongest edge point in the neighborhood of the user-selected point. This strongest edge point (i.e., the starting point) is found by searching over a 5 × 5 pixel square area with the manually selected point in its center. Once the algorithm has found the starting point, the program, searching in all directions and choosing the strongest one, finds the next contour point, which must have at least as strong a gradient as the starting point. The program then traces a contour from the most recent point, following the same principle described above; the contour is complete when it traces back to the starting point. The MS lesions detected are recorded in a file as regions of interest (ROIs) and superimposed on each image slice. The program automatically calculates the single ROI area. Manual outlining is required to modify part of the boundary of poorly defined lesions or (more rarely) to fully outline lesions not definable by contouring. The total lesion volume is then calculated, multiplying the total ROI area by the slice thickness. For the whole measurement process, the technicians followed recently published guidelines.[31]

Statistical analyses. Efficacy and safety analyses were based on the population of patients who received at least one dose of study medication and had available data. For efficacy variables, all hypothesis tests were carried out two-sided, with a significance level of <0.05 considered to be statistically significant.

The designated primary efficacy parameter was mean change in EDSS score from baseline to the final evaluation. Secondary clinical outcome measures were mean change from baseline in SNRS score and time to progression of MS. Disease progression was defined as an increase in EDSS score of ≥ 1.0 for patients with a baseline disability of 3.0 to 5.0 and an increase in EDSS score of ≥ 0.5 for patients with a baseline disability of 5.5 to 6.5, which was confirmed at the next scheduled visit. EDSS and SNRS examinations were performed by the blinded examining physician every second month during the double-blind phase. Treatment differences for the change from baseline to the final evaluation for these variables were assessed using a Wilcoxon's rank sum test. Comparisons were made between the placebo and cladribine 2.1 mg/kg groups and the placebo and cladribine 0.7 mg/kg groups, respectively. Time to progression of MS was analyzed using survival analysis methods. Kaplan-Meier estimates for the probabilities of failure were computed for each group. Log-rank tests were used to compare the distributions between the placebo and cladribine 2.1 mg/kg groups and between the placebo and cladribine 0.7 mg/kg groups.

The evaluation of MRI efficacy is based on the proportion of patients with contrast-enhanced T1-weighted brain lesions at the final evaluation. Additional MRI efficacy assessments are based on the number and volume of enhanced T1-weighted lesions and volume of T2-weighted lesions. Comparisons between treatment groups (placebo versus cladribine 2.1 mg/kg, placebo versus cladribine 0.7 mg/kg) of the proportion of patients with enhanced T1 lesions at months 6 and 12 and the final evaluation were made using Fisher's exact test. Treatment differences in enhanced T1 lesion volume and number, T2 lesion volume, and change and percent change in T2 lesion volume from baseline to final evaluation were assessed using Wilcoxon's rank sum test.

Safety analyses included summaries of adverse events. For laboratory analytes, vital signs, and body weights, means and mean changes from baseline were computed at each monthly visit.

Results. *Demographic and baseline characteristics.* The 159 eligible patients were randomly assigned to receive placebo (n = 54), cladribine 0.7 mg/kg (n = 53), or cladribine 2.1 mg/kg (n = 52). The three treatment groups

***Table 1** Demographic and baseline characteristics*

Characteristic	Placebo (n = 54)	Cladribine 0.7 mg/kg (n = 53)	Cladribine 2.1 mg/kg (n = 52)
Age, mean (y)	44.2	44.6	43.8
% Male/female	37/63	42/58	50/50
Pattern of disease			
% PPMS	26	36	29
% SPMS	74	64	71
Duration of disease (y)			
Mean	12.3	10.9	10.6
Median	11.7	10.0	8.8
EDSS score at entry			
Mean	5.6	5.6	5.6
Median	6.0	6.0	6.0
Category, % 3.0–5.0/5.5–6.5	31/69	30/70	25/75
SNRS score at entry			
Mean	60.9	60.7	62.3
Median	62.0	62.0	62.0

PPMS = primary progressive MS; SPMS = secondary progressive MS; EDSS = Expanded Disability Status Scale; SNRS = Scripps Neurological Rating Scale.

were similar with respect to age, gender, duration and pattern of disease, and baseline disability as defined by EDSS or SNRS scores (table 1). Overall, the median age was 44 years; 43% of patients were men and 57% were women. At baseline, 111 (70%) patients had SPMS and 48 (30%) patients had PPMS; 71% of patients had a baseline EDSS score of ≥5.5, indicating a population with substantial disability. Consistent with a population of more advanced disease and 30% of patients with PPMS, 63% had no enhanced lesions at baseline. Mean enhanced T1 lesion count was 1.3, and mean enhanced T1 lesion volume was 216.4 μL at baseline. Mean T2 lesion volume at baseline was 12.0 mL. Patients in the placebo group had a somewhat smaller mean enhanced T1 lesion volume than patients in the two cladribine groups (p = NS), and T1 lesion volumes at baseline had higher standard deviations among the cladribine patients than among the placebo patients.

Compliance. All 159 patients randomized to receive double-blind therapy received at least one dose of the study drug, and all are included in the efficacy analysis; 155 (97%) patients completed the double-blind phase. There were no withdrawals due to adverse events; 4 (3%) patients withdrew voluntarily from the study (subject choice) before completion of the double-blind phase (three from the low-dose cladribine group and one from the higher-dose group). The majority of patients received all eight scheduled courses of therapy (7/54 placebo-treated patients, 11/53 cladribine 0.7 mg/kg-treated patients, and 16/52 of 2.1 mg/kg-treated patients received a placebo substitution). The most common reasons for failure of the dosing criteria were fluctuations in hemoglobin levels and platelet counts, which occurred at a similar frequency in all groups.

Post-double-blind follow-up data are available for 148 of the 159 patients enrolled in the double-blind phase of this

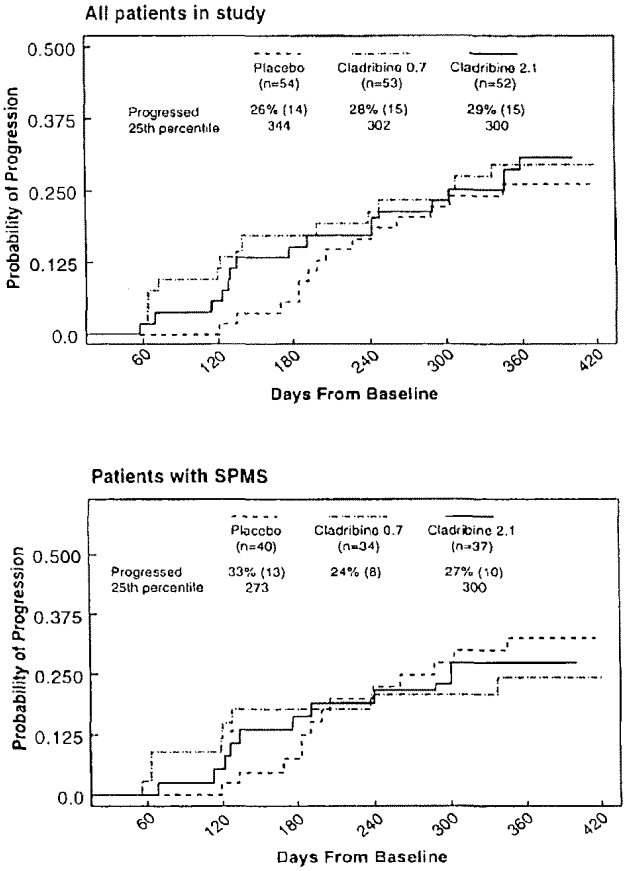

Figure 1. Probability of disease progression over time. SP = secondary progressive.

ongoing study. For the outcomes presented here, the mean duration of follow-up from the first dose was 29 months.

Clinical outcomes. During the 12-month double-blind phase, the mean changes in EDSS and SNRS scores from baseline to final evaluation were small in all three treatment arms (placebo, 0.7 mg/kg, and 2.1 mg/kg cladribine), and no differences among treatment groups were observed for placebo and cladribine. Examination of changes in EDSS scores according to pattern of disease showed that for patients with SPMS, EDSS scores increased modestly (0.3) over time in the placebo group but less in the active treatment groups (±0.0, p = NS); by comparison, very little change in EDSS score was experienced in any treatment arm by patients with PPMS. Similarly, although no significant differences among treatment groups were found in time to progression assessed by Kaplan-Meier estimate for all patients, there was a trend toward a more favorable clinical response to cladribine than to placebo in the SPMS subgroup (figure 1); 33% of patients in the placebo group met the criteria for disease progression by the end of the double-blind phase, compared with 24% to 27% of cladribine-treated patients with SPMS.

Exacerbations, steroid utilization, and hospitalizations did not differ among the three groups.

Follow-up EDSS scores obtained after the 12-month double-blind phase, but before retreatment, are available

Table 2 Summary of MRI outcomes during double-blind phase of study: all patients

MRI parameter	Placebo n (%) or mean (SD)	Cladribine 0.7 mg/kg n (%) or mean (SD)	*p* Value	Cladribine 2.1 mg/kg n (%) or mean (SD)	*p* Value
Enhanced T1 lesions					
Proportion of patients with lesions (%)[a,b,d]					
Baseline	53 (38%)	52 (33%)		50 (36%)	
Month 6	51 (33%)	49 (12%)	0.0169	52 (2%)	0.001
Month 12	50 (32%)	48 (10%)	0.0131	48 (6%)	0.0017
Final evaluation	54 (31%)	51 (10%)	0.0080	52 (6%)	0.0009
Mean number of lesions (SD)[a,c,d]					
Baseline	1.17 (2.23)	1.64 (4.43)		1.10 (2.07)	
Month 6	0.78 (1.49)	0.17 (0.52)	0.008	0.12 (0.85)	<0.001
Month 12	0.57 (1.10)	0.13 (0.40)	0.007	0.09 (0.35)	0.001
Final evaluation	0.58 (1.12)	0.12 (0.39)	0.005	0.08 (0.34)	0.001
Mean volume of lesions in μL (SD)[a,c,d]					
Baseline	142.66 (302.15)	283.82 (803.10)		235.24 (777.94)	
Month 6	78.67 (168.07)	12.44 (44.35)	0.008	19.40 (137.18)	<0.001
Month 12	67.76 (119.65)	10.94 (39.99)	0.005	6.36 (26.63)	0.001
Final evaluation	78.11 (155.74)	10.28 (38.83)	0.003	5.98 (25.85)	0.001
T2 lesions					
Mean lesion volume (mL) (SD)[a,c,d]					
Baseline	12.90 (12.35)	13.03 (12.37)		9.91 (8.50)	
Month 6	13.45 (12.77)	13.15 (12.09)	0.872	9.78 (8.60)	0.155
Month 12	13.13 (13.11)	12.62 (11.52)	0.944	9.79 (8.80)	0.231
Final evaluation	13.31 (13.00)	12.65 (11.96)	0.868	9.71 (8.56)	0.180
Change from baseline to final evaluation[c,d,e]					
Mean (SD)	0.41 (1.72)	−0.39 (1.70)		−0.20 (1.13)	
Median	0.10	−0.01	0.055	−0.13	0.040
Percent change from baseline to final evaluation[c,d,e]					
Mean (SD)	1.81 (11.38)	−1.67 (14.98)		−3.93 (14.80)	
Median	1.53	0.03	0.144	−2.51	0.029

[a] Includes patients with both baseline and final evaluations.
[b] Fisher's exact test (two-sided significance).
[c] Based on Wilcoxon's (Mann-Whitney) rank sum test.
[d] The final evaluation is the last evaluation for each patient up to month 12 during year 1.
[e] Positive change indicates disease progression.

through month 24 for a sizable cohort of patients, although cohort sizes became smaller as some patients entered retreatment during the follow-up phase. Although mean EDSS scores increased over time in all treatment groups, scores for the follow-up period were also analyzed by pattern of disease. For patients with SPMS, mean changes in EDSS scores were somewhat more favorable with cladribine (0.2 and 0.3, respectively, for the 0.7-mg/kg and 2.1-mg/kg doses) compared with placebo (0.6) by 24 months. No difference was observed for patients with PPMS.

Magnetic resonance outcomes. Proportion of patients with enhanced T1 lesions. At baseline, approximately 35% of patients in each treatment group had enhanced T1 lesions (figure 2, table 2). Whereas the proportion of patients with enhanced T1 lesions remained nearly unchanged from baseline to final evaluation in the placebo group, the proportion of cladribine-treated patients with enhanced T1 lesions decreased significantly, to 10% in the 0.7 mg/kg group ($p = 0.0080$) and 6% in the 2.1 mg/kg group ($p = 0.0009$). By final evaluation, there was a 70% reduction in the proportion of patients with enhanced T1 lesions in the cladribine 0.7 mg/kg group and an 83% reduction in this proportion in the cladribine 2.1 mg/kg group, compared with a reduction of 18% in the placebo group. The difference between the cladribine and placebo groups in the proportion of patients with enhanced T1 lesions was statistically significant at month 6 (see figure 2, table 2). It remained significant through month 18 for the 0.7 mg/kg dose and through month 24 for the 2.1 mg/kg dose (table 3).

Subgroup analysis of the proportion of patients with enhanced T1 lesions by pattern of disease showed no sig-

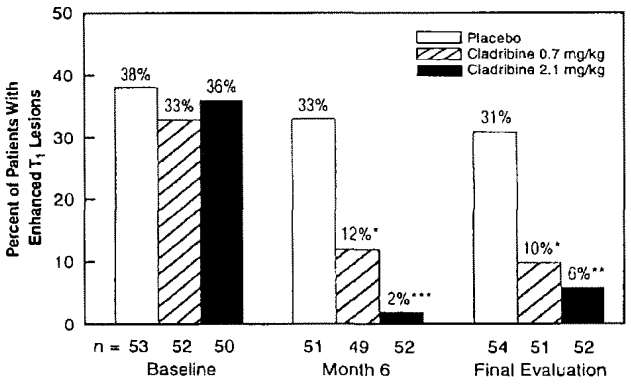

*Figure 2. Proportion of patients with enhanced T1 lesions during the double-blind phase study. *p < 0.02 versus placebo. **p < 0.001 versus placebo. ***p < 0.0001 versus placebo.*

nificant difference among treatment groups in patients with PPMS (data not shown). In patients with SPMS, however, significantly smaller proportions of patients treated with either cladribine dose had enhanced T1 lesions at month 6 and the double-blind final evaluation, and those treated with 2.1 mg/kg maintained significant differences at follow-up months 18 and 24 (data not shown).

Examination of the relationship between the status of patients with and without enhanced T1 lesions at baseline and their status at final evaluation showed that for patients who presented without enhanced T1 lesions at baseline, new enhanced T1 lesions developed by final evaluation in 18% of placebo patients compared with 9% and 6%, respectively, of the low- and high-dose cladribine groups (NS). Moreover, for patients with enhanced T1 lesions present at baseline, the treatment effect on enhanced T1 lesions at final evaluation was significantly greater in patients receiving 0.7 mg/kg ($p < 0.02$) and 2.1 mg/kg ($p < 0.002$) cladribine than in the placebo patients; these differences in treatment effect between the placebo group

Table 3 *Summary of MRI outcomes during post–double-blind follow-up: all patients*

	Placebo	Cladribine 0.7 mg/kg		Cladribine 2.1 mg/kg	
MRI parameter	n (%) or mean (SD)	n (%) or mean (SD)	*p* Value	n (%) or mean (SD)	*p* Value
Enhanced T1 lesions					
Proportion of patients with lesions (%)[a–c]					
Baseline	14 (36%)	15 (32%)		16 (36%)	
Final evaluation	14 (36%)	5 (10%)	0.0079	2 (4%)	0.0002
Month 18	14 (36%)	5 (11%)	0.0089	1 (2%)	0.0001
Month 24	7 (24%)	4 (11%)	0.1965	0 (0%)	0.0014
Mean number of lesions (SD)[a,c,d]					
Baseline	0.64 (1.04)	1.72 (4.56)		1.09 (2.13)	
Final evaluation	0.62 (1.14)	0.13 (0.40)	0.004	0.04 (0.21)	<0.001
Month 18	0.62 (1.37)	0.20 (0.67)	0.011	0.07 (0.46)	<0.001
Month 24	1.17 (3.97)	0.26 (0.82)	0.182	0.0 (0.00)	0.001
Mean volume of lesions (μL)[a,c,d]					
Baseline	83.10 (160.33)	298.11 (826.49)		241.36 (816.41)	
Final evaluation	75.87 (126.94)	10.94 (39.99)	0.003	3.20 (15.10)	<0.001
Month 18	111.59 (351.47)	21.16 (90.58)	0.006	4.42 (28.97)	<0.001
Month 24	168.83 (708.80)	69.40 (236.50)	0.238	0.00 (0.00)	0.001
T2 lesions					
Mean lesion volume (mL) (SD)[a,c,d]					
Baseline	10.42 (8.80)	13.28 (12.49)		10.34 (8.81)	
Final evaluation	10.47 (8.71)	12.87 (12.06)	0.395	10.08 (8.87)	0.825
Month 18	10.50 (8.75)	13.22 (12.21)	0.379	9.91 (8.29)	0.769
Month 24	10.75 (9.55)	12.41 (12.95)	0.839	10.36 (8.83)	0.945
Percent change from baseline to Month 24[c,e]					
Mean (SD)	3.74 (15.38)	1.02 (23.16)		−4.22 (17.55)	

[a] Includes patients with both baseline and final evaluations.
[b] Fisher's exact test (two-sided significance).
[c] The final evaluation is the last evaluation of the double-blind phase.
[d] Based on Wilcoxon's (Mann-Whitney) rank sum test.
[e] Positive change indicates disease progression.

and cladribine groups were statistically significant at month 6.

Volume and number of enhanced T1 lesions. The cladribine groups had approximately 90% reductions in the mean number of enhanced T1 lesions at month 6 and maintained 92% reductions through final evaluation, compared with 33% and 50% reductions in the placebo group at month 6 and final evaluation, respectively (see table 2). The differences in the numbers of these lesions at the final evaluation between the placebo and cladribine 0.7 mg/kg groups ($p = 0.005$) and the placebo and cladribine 2.1 mg/kg groups ($p = 0.001$) were statistically significant, as were the differences at month 6. Compared with a 3% reduction in the mean number of enhanced T1 lesions in the placebo group at month 18 and a 77% increase at month 24, the cladribine groups maintained a 91% reduction at month 18 ($p < 0.001$) and month 24 ($p = 0.005$, see table 3).

The mean volume of enhanced T1 lesions also decreased from baseline in all three treatment groups during double-blind therapy, with greater reductions observed in the two cladribine groups (96% and 97%, respectively, for the low- and high-dose groups) compared with the placebo group (45%; see table 2). Differences between placebo and cladribine treatments in enhanced T1 lesion volume were statistically significant at each timepoint after baseline, with >90% reduction in both cladribine treatment groups at month 6. Compared with 34% and 70% increases in the volume of enhanced T1 lesions in the placebo group at months 18 and 24, respectively, patients receiving cladribine had a 95% reduction in volume at month 18 ($p < 0.001$) and an 87% reduction at month 24 ($p = 0.007$, see table 3).

Subgroup analysis of the volume and number of enhanced T1 lesions by pattern of disease and post-double-blind follow-up data are consistent with the observations on proportions of patients having such lesions and support the finding that the effect of cladribine on suppression of enhanced T1 lesions is greater in patients with SPMS and is sustained for up to 24 months, particularly at the 2.1 mg/kg dose.

Volume of T2 lesions. Mean baseline T2 lesion volumes were generally comparable across all treatment groups. During the double-blind phase, both cladribine groups had a slight decrease in mean T2 lesion volume from baseline to final evaluation (−0.39 mL for the cladribine 0.7 mg/kg group and −0.20 mL for the cladribine 2.1 mg/kg group; the placebo group showed a mean increase of 0.41 mL) (see table 2). The change from baseline to final evaluation between the placebo group and the cladribine 2.1 mg/kg group was statistically significant ($p = 0.040$), indicating that lesion load did not accumulate as rapidly in the high-dose cladribine group.

In the placebo group, there was a median percent increase of 1.53% in T2 lesion volume from baseline to final evaluation (see table 2). Cladribine-treated patients, however, had a small, dose-dependent, median percent decrease in T2 lesion volume during the double-blind phase of the study, suggesting a stabilization of T2 lesion accumulation by the end of 1 year following the start of treatment (figure 3). The difference in median percent change in T2 lesion volume for the cladribine 2.1 mg/kg group was

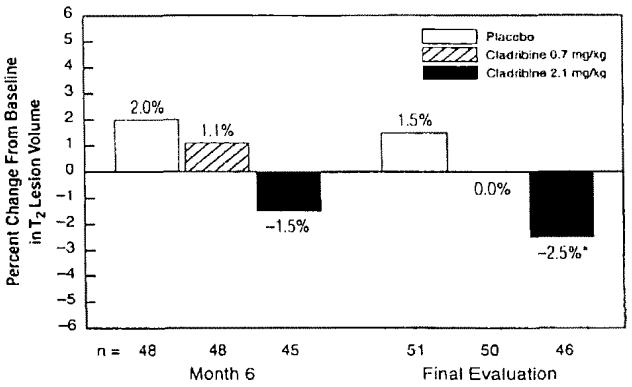

Figure 3. Change in T2 lesion volume over time during the double-blind phase of the study. *p = *0.029 versus placebo.*

significantly different from that for placebo treatment ($p = 0.029$).

Among patients with available data during the second year of follow-up, the mean percent change in T2 lesion volume remained negative at −4.2% in patients treated with 2.1 mg/kg cladribine, whereas the placebo group continued to show an increase of 3.7% and the 0.7 mg/kg group showed a slight increase of 1.0% (see table 3). Subgroup analysis showed that the treatment effect of cladribine on median percent change in T2 lesion volume was significant over 24 months of follow-up in patients with SPMS but not in those with PPMS (data not shown). Moreover, the decrease in T2 lesion volume, which appeared to be dose related in the overall study analysis, was independent of dose in patients with SPMS.

Safety evaluation. All 159 patients were included in the safety analyses. Most patients within each treatment group received the maximum assigned total cladribine doses. Cladribine was generally well tolerated, and 97% of patients completed the 12-month double-blind phase of the study. There were no drop-outs due to adverse events.

Adverse events. A majority of reported adverse events occurred with comparable frequency in patients who received placebo and patients treated with cladribine. Most of the adverse events in all three treatment groups were mild or moderate in severity, not treatment limiting, and were judged by the investigator to be unlikely to be related to study drug therapy. Many of the frequently reported adverse events, such as pain, urinary tract infection, and injury, were often related to the underlying disease, and the various application site disorders were related to subcutaneous injection of the study medication.

The most common treatment-emergent adverse events (reported for ≥10% of patients in any treatment group), whose frequency was at least 5% higher among patients in one or both cladribine groups relative to the placebo group, are listed in table 4. Muscle weakness, hypertonia, purpura, rhinitis, and ataxia occurred more frequently among cladribine-treated patients than in the placebo group. Injection site pain, injury, dizziness, and tremor were reported more frequently in the placebo group than in cladribine-treated patients. Upper respiratory tract infection, pharyngitis, back pain, arthralgia, and skin disorder were more common in patients in the cladribine

Table 4 *Treatment-emergent adverse events seen more frequently in cladribine-treated patients**

Preferred term	Placebo, n = 54	Cladribine 0.7 mg/kg, n = 53	Cladribine 2.1 mg/kg, n = 52
Upper respiratory tract infection	16 (30)	13 (25)	23 (44)
Muscle weakness	3 (6)	10 (19)	11 (21)
Purpura	5 (9)	8 (15)	13 (25)
Injection site reaction	9 (17)	7 (13)	13 (25)
Hypertonia	6 (11)	9 (17)	10 (19)
Back pain	7 (13)	5 (9)	11 (21)
Urinary tract infection	6 (11)	10 (19)	6 (12)
Depression	9 (17)	12 (23)	4 (8)
Arthralgia	4 (7)	4 (8)	7 (13)
Rhinitis	2 (4)	5 (9)	5 (10)
Ataxia	2 (4)	4 (8)	5 (10)
Pharyngitis	2 (4)	1 (2)	8 (15)

Values are n (%).

* Adverse events reported by ≥10% of the patients in any one of the three treatment groups and ≥5% more patients in a cladribine group than in the placebo group.

2.1 mg/kg group than in those receiving cladribine 0.7 mg/kg or placebo.

Among the less common but clinically important treatment-emergent adverse events, the incidence of infections not usually seen in the MS population was similar in the three treatment groups. One patient in each treatment group experienced herpes zoster infection of marked severity; in two of these patients, one receiving placebo and the other 0.7 mg/kg cladribine, the infection contributed to discontinuation of the study drug, and all three cases resolved with antiviral treatment. Herpes simplex infection occurred in 3 (6%) placebo-treated patients and in 1 (2%) patient in the cladribine 0.7 mg/kg group.

Clinical laboratory analytes. *Hematologic changes.* At the final evaluation of the double-blind period, dose-related decreases in mean leukocyte count, absolute lymphocyte count, absolute neutrophil count, absolute monocyte count, platelet count, hemoglobin, and hematocrit were observed in cladribine-treated patients, compared with small increases or no change in the mean values of these analytes in the placebo group. Consistent with the expected pharmacologic activity of cladribine, the largest observed effect was a suppression in lymphocyte count. The mean lymphocyte count was suppressed at both dosage levels of cladribine from the first cycle of treatment throughout the double-blind observation phase, and remained below the normal range for this analyte over time in the 2.1 mg/kg group (figure 4). The observed decreases in the other analytes were modest, and the mean values were within the normal range at the final visit. Two patients (one receiving placebo and the other high-dose cladribine) had short-lived thrombocytopenia (platelet counts ≤ 100 × 10^9/L), and one patient in the cladribine 2.1 mg/kg group had a single low neutrophil count (960/μL).

Lymphocyte subset analyses showed cladribine treatment to be associated with dose-dependent decreases in

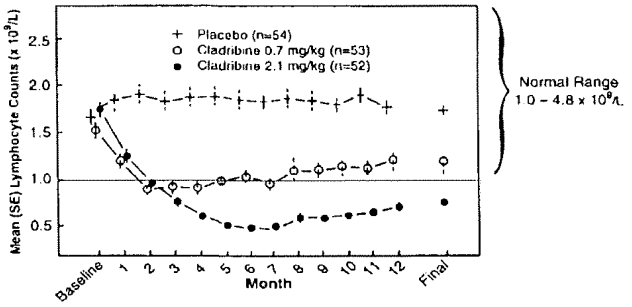

Figure 4. Change in mean lymphocyte count over time during the double-blind phase of the study.

mean levels of $CD4^+$, $CD3^+$, $CD8^+$, and to a lesser degree, $CD19^+$ lymphocytes. More patients in the 2.1 mg/kg group experienced $CD4^+$ counts ≤ 200/μL (n = 31) than did those in the 0.7 mg/kg (n = 5) or placebo (n = 1) groups; two patients in the high-dose group had at least one $CD4^+$ count ≤ 50/μL. In addition, there was a dose-dependent decrease in the mean $CD4^+/CD8^+$ ratio among cladribine-treated patients compared with a small increase among placebo-treated patients. Although both $CD4^+$ and $CD8^+$ lymphocyte counts showed a dose-related suppression among cladribine-treated patients, the observed decrease in the $CD4^+/CD8^+$ ratio indicated a more pronounced effect on the $CD4^+$ subset. Subset analysis also revealed a modest and transient dose-dependent increase in the mean percentage of $CD16^+$ plus $CD56^+$ lymphocytes. The relatively minor, transient effect on $CD19^+$ lymphocytes and the effect on $CD16^+$ plus $CD56^+$ lymphocytes may explain the relatively low number of serious infections in the presence of significant overall lymphocytopenia.

Serum chemistry. Examination of mean changes from baseline to final evaluation during the double-blind phase revealed no treatment-related effects on hepatic or renal function tests or other serum chemistries.

Physical findings. Mean changes from baseline values for vital signs, body weight, physical findings, and ECG were small and clinically insignificant.

Discussion. Using EDSS scores as an outcome measure, no significant treatment effects were found for cladribine in this double-blind study of patients with progressive MS. The lack of overall treatment difference in this study is likely due to the majority of patients having relatively high EDSS scores at baseline (median score for all three treatment groups, 6.0) and the placebo group having only a modest increase from baseline in mean EDSS score. Multivariate predictive models of short-term and long-term worsening on the EDSS and meta-analysis of placebo-treated control groups in progressive MS have shown that the spectrum of disability is bimodal, with one peak at EDSS 1 to 3 and another at 6.[32] Patients with a baseline EDSS score of 3.0 to 5.0 are much more likely to deteriorate by 1 full point on the EDSS in a shorter period of time than are those with a baseline score ≥ 6.0.[33,34]

The statistical sizing and duration of the double-blind phase of this study were based on the assump-

tion that placebo-treated patients with progressive MS would show a greater degree of disease progression over the course of 12 months and did not anticipate enrollment of mainly patients with more severe disability. The trial was underpowered for the patient population enrolled. In addition, patients with PPMS may be more resistant to therapy than those with SPMS, and approximately one third of the patients enrolled in this study had PPMS. Subgroup analysis by disease pattern suggested stabilization of disability in cladribine-treated patients with SPMS at final evaluation but not in those with PPMS. Long-term follow-up through month 24 showed a trend toward a beneficial cladribine effect in the SPMS subgroup at both the 0.7 and 2.1 mg/kg doses.

Because clinical efficacy was not shown in this trial, it is necessary to scrutinize the Scripps study for type I errors (false positives). Sources of concern in the Scripps trial[25] include the replacement of cladribine dropouts in a small crossover trial. An intent-to-treat analysis, which would have included data from these patients, is mentioned but not reported in detail. The disability scores upon which efficacy was determined were not "confirmed" by a definition of sustained worsening over the standard 3- to 6-month periods used in other trials. The use of means of ordinal scores (e.g., the Kurtzke scale) as an outcome measure is problematic. In the second year of the study, 5/24 patients destined to receive placebo in the crossover limb actually received one dose of cladribine. Rapid worsening of the placebo group presaged the ultimate failure of the trial's reproducibility. This type of placebo-group worsening has been the Achilles heel of many previous studies. The late (month 27 to 30) worsening of patients who received cladribine initially, although perhaps contaminated by small numbers and examiner unblinding (which should have mitigated the effect), suggested that the early treatment effect was not durable.[26]

The MRI findings, however, were more compelling. Gadolinium-enhanced T1 lesions represent areas of breakdown in the blood–brain barrier and are generally believed to be sites of new inflammation that probably precede symptoms and other MRI signs in MS.[35] Gadolinium-enhancing lesions were virtually eliminated by treatment with cladribine. Both the 0.7 mg/kg and 2.1 mg/kg doses were significantly superior to placebo with respect to the proportion of patients having detectable enhanced T1 lesions as well as to the mean volume and number of such lesions at 6 months and final evaluation. Moreover, statistically significant differences were maintained through month 18 for the 0.7 mg/kg dose and through month 24 for the 2.1 mg/kg dose. These MRI outcomes compare favorably with those in a recently published 2-year study of interferon β-1b in the treatment of patients with SPMS (mean baseline EDSS score, 5.1 ± 1.1),[14] and are supported by similar observations from two previous studies with cladribine treatment in patients with SPMS (median EDSS score, 4.0)[25,26] and in patients with RRMS (median EDSS score, 3.5).[36]

Although it is conceivable that events related to progression are different from those involved with the inflammation identified by gadolinium enhancement, the MRI changes in this study were robust. Longitudinal natural history studies in MS patients suggest that clinical and radiologic worsening over the short term can be predicted by gadolinium enhancement and by new T2 lesions.[37] However, enhancement does not appear to predict the development of long-term disability in MS, at least in studies of 2 years' duration.[22]

Cladribine doses of 0.7 mg/kg and 2.1 mg/kg were well tolerated. The most common adverse events were related to the patients' underlying disease or to SC administration of the study drug. They were mild or moderate in severity, not treatment limiting, and were judged by the investigator to be unrelated to study drug therapy. There were no systemic constitutional signs and symptoms that would have led to unblinding. Cells of most hematologic lineages showed modest dose-related suppression following cladribine treatment but means were within the normal range; this is an expected consequence of the selective lymphocytotoxic effects of the drug. A more marked, long-lasting, dose-related lymphocyte count reduction confirmed the pharmacologic activity of cladribine in this study. This has persisted beyond 2 years in several patients. Herpes infections occurred rarely, with similar frequencies in the three treatment groups, and resolved with appropriate medical care. There were no serious infections in this group of patients; the risk of potentially serious infections not commonly encountered in patients with MS appears to be higher in patients with SPMS receiving cladribine at a dose of 2.8 mg/kg.[25,26]

Although serious neurologic, hepatic, and renal adverse events have been observed in cancer patients receiving cladribine at doses considerably higher than those administered to patients with MS,[24,38] no serious adverse events could be attributed to cladribine treatment in this study. There were no treatment-related deaths, no suicides or suicide attempts, and the incidence of depression was considerably lower in patients treated with 2.1 mg/kg than in the other two groups.

The cladribine doses used in the current study were chosen to reduce the hematopoietic effects of the drug reported in the previous trial, in which a total dose of 2.8 mg/kg was administered.[25,26] The high dose in the current study (2.1 mg/kg) suppressed the mean lymphocyte count by 60%, compared with 70% in the previous study. The intensity of the dosing schedule differed in the two trials (0.07 mg/kg/day versus 0.1 mg/kg/day; 0.35 mg/kg/5-day cycle versus 0.7 mg/kg/7-day cycle), which may have contributed to the improved tolerability and lower incidence of infections in this study.

Acknowledgment

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Appendix 1

The Cladribine Clinical Study Group comprises the following participating research centers, principal investigators (in italics), and investigative teams: University Hospital, London, Ontario, Canada—*George P. A. Rice, MD,* George Ebers, MD, Kang Howson-Jan, MD, M. Vandervoort, RN, L. Froste, RN, W. Koopman; Oregon Health Sciences University, Portland, OR—*Dennis N. Bourdette, MD,* James J. Cereghino, MD, Michele K. Mass, MD, Joseph Quinn, MD, Gerald M. Segal, MD, Ruth H. Whitham, MD; Yale University School of Medicine, New Haven, CT—*Joseph B. Guarnaccia, MD,* Brian Smith, MD, Jonathan M. Goldstein, MD, Henry M. Rinder, MD; University of Medicine and Dentistry of New Jersey, Newark, NJ—*Stuart D. Cook, MD,* Shalini Bansil, MD, Mary Ann Picone, MD; Allegheny University of the Health Sciences, Philadelphia, PA—*Fred D. Lublin, MD;* Thomas Jefferson University, Philadelphia, PA—*Robert L. Knobler, MD;* University of British Columbia, Vancouver, Canada—*Joel Oger, MD,* Stanley A. Hashimoto, MD, Donald W. Paty, MD; The R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ—James Baldassarre, MD, Liang Xiu, PhD.

The Cladribine MRI Study Group consists of the following investigators: Neuroimaging Research Unit, Dept of Neuroscience, Scientific institute Ospedale San Raffaele, University of Milan, Italy—*Massimo Filippi, MD,* Giovanna Mastronardo, MD, Marco Rovaris, MD; Clinical Trials Unit, Dept of Neuroscience, Scientific Institute Ospedale San Raffaele, University of Milan, Italy—*Giancarlo Comi, MD.*

The Independent Safety Monitoring Board was composed of N. Fishman, MD, University of Pennsylvania, Philadelphia; R. Hemdon, MD, Department of Veterans Affairs, Jackson, MS; S. van den Noort, MD, University of California, Irvine; K. Rai, MD, Long Island Jewish Medical Center, New Hyde Park, NY; N. Temkin, MD, Harborview Medical Center, Seattle, WA; W. Tourtellotte, MD, PhD, Veterans Affairs Medical Center, Los Angeles, CA.

Appendix 2

Hematologic dosing criteria

1. Platelet count:
   $\geq 200 \times 10^9$/L *or*
   if $150–200 \times 10^9$/L, not <50% of previous count *or*
   if $125–150 \times 10^9$/L, not <80% of previous count
2. Absolute neutrophil count $> 1.0 \times 10^9$/L
3. Hemoglobin:
   no decline >1.5 g/dL from previous monthly value *or*
   no decline >3 g/dL from baseline value

References

1. Anderson DW, Ellenberg JH, Leventhal CM, Reingold SC, Rodriguez M, Silberberg DH. Revised estimate of the prevalence of multiple sclerosis in the United States. Ann Neurol 1992;31:333–336.
2. Weinshenker BG. Natural history of multiple sclerosis. Ann Neurol 1994;36:S6–S11.
3. Goodkin DE. Interferon-β therapy for multiple sclerosis. Lancet 1998;352:1486–1487.
4. Jacobs LD, Cookfair DL, Rudick RA, et al. A phase III trial of intramuscular recombinant interferon beta as treatment for exacerbating-remitting multiple sclerosis: design and conduct of study and baseline characteristics of patients. Multiple Sclerosis Collaborative Research Group (MSCRG). Mult Scler 1995;1:118–135.
5. Jacobs LD, Cookfair DL, Rudick RA, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. Ann Neurol 1996;39:285–294.
6. Rudick RA, Goodkin DE, Jacobs LD, et al. Impact of interferon beta-1a on neurologic disability in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). Neurology 1997;49:358–363.
7. Simon JH, Jacobs LD, Campion M, et al. Magnetic resonance studies of intramuscular interferon β-1a for relapsing multiple sclerosis. Ann Neurol 1998;43:79–87.
8. Fieschi C, Pozzilli C, Bastianello S, et al. Human recombinant interferon beta in the treatment of relapsing-remitting multiple sclerosis: preliminary observations. Mult Scler 1995; 1(suppl 1):S28–S31.
9. Pozzilli C, Bastianello S, Koudriavtseva T, et al. Magnetic resonance imaging changes with recombinant human interferon-β-1a: a short term study in relapsing-remitting multiple sclerosis. J Neurol Neurosurg Psychiatry 1996;61:251–258.
10. Paty DW, Blumhardt LD, on behalf of the transitional PRISMS Study Group. High-dose subcutaneous interferon β-1a is efficacious in transitional multiple sclerosis, a group at high risk for progression in disability. Ann Neurol 1998;44: 503. Abstract W240.
11. The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993;43:655–661.
12. Paty DW, Li DKB, the UBC MS/MRI Study Group, the IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993;43:662–667.
13. The IFNB Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group. Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. Neurology 1995;45:1277–1285.
14. European Study Group on Interferon β-1b in Secondary Progressive MS. Placebo-controlled multicentre randomised trial of interferon β-1b in treatment of secondary progressive multiple sclerosis. Lancet 1998;352:1491–1497.
15. Miller DH, Albert PS, Barkhof F, et al. Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. Ann Neurol 1996;39:6–16.
16. McFarland HF, Frank JA, Albert PS, et al. Using gadolinium-enhanced magnetic resonance imaging lesions to monitor disease activity in multiple sclerosis. Ann Neurol 1992;32:758–766.
17. Smith ME, Stone LA, Albert PS, et al. Clinical worsening in multiple sclerosis is associated with increased frequency and area of gadopentetate dimeglumine-enhancing magnetic resonance imaging lesions. Ann Neurol 1993;33:480–489.
18. Stone LA, Smith ME, Albert PS, et al. Blood–brain barrier disruption on contrast-enhanced MRI in patients with mild relapsing-remitting multiple sclerosis: relationship to course, gender, and age. Neurology 1995;45:1122–1126.
19. Koudriavtseva T, Thompson AJ, Pozzilli C, et al. Role of a baseline scan in predicting clinical and MRI activity of relapsing-remitting MS patients. J Neuroimmunol 1995; 61(suppl 1):41. Abstract.
20. Filippi M, Horsfield MA, Tofts PS, Barkhof F, Thompson AJ, Miller DH. Quantitative assessment of MRI lesion load in monitoring the evolution of multiple sclerosis. Brain 1995;118: 1601–1612.
21. Filippi M, Horsfield MA, Morrissey SP, et al. Quantitative brain MRI lesion load predicts the course of clinically isolated syndromes suggestive of multiple sclerosis. Neurology 1994; 44:635–641.
22. Kappos L, Moere EW, Schoetzau A, et al. Predictive value of magnetic resonance imaging for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis. Lancet 1999;353:964–968.
23. Evans AC, Frank JA, Antel J, Miller DH. The role of MRI in clinical trials of multiple sclerosis: comparison of image processing techniques. Ann Neurol 1997;41:125–132.
24. Beutler E. Cladribine (2-chlorodeoxyadenosine). Lancet 1992; 340:952–956.
25. Sipe JC, Romine JS, Koziol JA, McMillan R, Zyroff J, Beutler E. Cladribine in treatment of chronic progressive multiple sclerosis. Lancet 1994;344:9–13.
26. Beutler E, Sipe JC, Romine JS, Koziol JA, McMillan R, Zyroff J. The treatment of chronic progressive multiple sclerosis with cladribine. Proc Natl Acad Sci USA 1996;93:1716–1720.
27. Schumacher GA, Beebe G, Kibler RF, et al. Problems of experimental trials of therapy in multiple sclerosis: report by the

panel on the evaluation of experimental trials of therapy in multiple sclerosis. Ann NY Acad Sci 1965;122:552–568.
28. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227–231.
29. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an Expanded Disability Status Scale (EDSS). Neurology 1983;33:1444–1452.
30. Barkhof F, Filippi M, van Waesberghe JH, et al. Improving interobserver variation in reporting gadolinium-enhanced MRI lesions in multiple sclerosis. Neurology 1997;49:1682–1688.
31. Filippi M, Gawne-Cain ML, Gasperini C, et al. Effect of training and different measurement strategies on the reproducibility of brain MRI lesion load measurements in multiple sclerosis. Neurology 1998;50:238–244.
32. Weinshenker BG, Bass B, Rice GPA, et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain 1989;112:133–146.
33. Weinshenker BG, Issa M, Baskerville J. Long-term, short-term outcome of multiple sclerosis: a 3-year follow-up study. Arch Neurol 1996;53:353–358.
34. Weinshenker BG, Issa M, Baskerville J. Meta-analysis of the placebo-treated groups in clinical trials of progressive MS. Neurology 1996;46:1613–1619.
35. Kermode AG, Thompson AJ, Tofts P, et al. Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis. Brain 1990;113:1477–1489.
36. Sipe JC, Romine JS, Koziol J, Zyroff J, McMillan R, Beutler E. Cladribine improves relapsing-remitting MS: a double blind, placebo controlled study. Neurology 1997;48(suppl 2):A340. Abstract S345.003.
37. Molyneux PD, Filippi M, Barkhof F, et al. Correlations between monthly enhanced MRI lesion rate and changes in T2 lesion volume in multiple sclerosis. Ann Neurol 1998;43:332–339.
38. Beutler E, Piro LD, Savan A. 2-Chlorodeoxyadenosine (2-CdA): a potent chemotherapeutic and immunosuppressive nucleoside. Leuk Lymphoma 1991;5:1–8.

A Wallerian degeneration pattern in patients at risk for MS

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Article abstract—*Background:* Demyelination alone may not explain the progressive disability that frequently develops in MS. An alternative explanation for irreversible disability assumes a contribution from axonal injury or loss. In theory, axonal injury may occur in the focal areas characterized by early inflammation, or can be more distant, as in Wallerian degeneration. However, Wallerian degeneration is thought of as a rare or a late finding in MS. *Methods:* Studies showing a classic Wallerian degeneration pattern in the corticospinal tract were selected from a review of MR studies from patients enrolled in a longitudinal treatment trial. Entry was based on first occurrence of an isolated neurologic syndrome consistent with MS and a positive MRI. *Results:* This report is based on five cases followed longitudinally who showed development of a classic T2-hyperintense lesion along the ipsilateral corticospinal tract, subsequent to an initial inciting event located in the white matter located in the superior aspect of the corona radiata. Lesions were evident as T2-hyperintensity persisting throughout the 12 to 18 months of observation. *Conclusions:* This series suggests that Wallerian degeneration, implying axonal injury, may occur as a sequela of acute demyelinating lesions in patients presenting with their first symptoms suggestive of MS. This can produce a component of the increasing burden of T2-hyperintense lesions temporally and spatially dissociated from inflammatory or demyelinating activity. Further studies are required to determine if Wallerian degeneration is an important factor contributing to disability progression in MS. **Key words:** Wallerian degeneration—MS.

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MS is understood to be a predominantly inflammatory and demyelinating disease with glial proliferation that is relatively sparing of axons. When described in MS, axonal injury has generally been considered a finding of late stages of disease, or as a consequence of relatively severe, but rare lesions.[1-3] As demyelination alone may not account for progressive disability in MS, interest has been focused re-

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A Double-Blind, Placebo-Controlled, Randomized Trial of Cladribine in Relapsing-Remitting Multiple Sclerosis

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We conducted an 18-month, placebo-controlled, double-blind study to evaluate cladribine in the treatment of 52 patients with relapsing-remitting multiple sclerosis. Patients received either placebo or cladribine 0.07 mg/kg/day by subcutaneous injection for 5 consecutive days as six monthly courses for a total cumulative dose of 2.1 mg/kg. Analysis of results revealed a statistically significant favorable effect of cladribine on the joint frequency and severity of relapses and magnetic resonance imaging (MRI) findings. MRI-enhancing lesions were completely suppressed in the cladribine patients by the sixth month of treatment. Mild segmental herpes zoster occurred in two cladribine-treated patients and one patient receiving placebo. Otherwise, there were no side effects or adverse events. We conclude that cladribine shows promise as a treatment for relapsing-remitting multiple sclerosis.

Although the cause and cure of multiple sclerosis (MS) remains unknown, it is generally accepted that immunological mechanisms play an important role in the inflammatory demyelination of the central nervous system that is the pathological hallmark of the disease. For this reason, current approaches to the treatment of MS patients are focused on modulation or suppression of the immune system.

The drug cladribine [2-chlorodeoxyadenosine (2-CdA), Mylinax, Ortho McNeil, Raritan, NJ] is a selective immunosuppressive molecule synthesized by Carson to mimic the immunodeficiency state seen in hereditary adenosine deaminase deficiency (1). It is a purine nucleoside with chlorine substituted for hydrogen at the 2 position of the purine ring. This makes the molecule resistant to adenosine deaminase, leading to accumulation of deoxynucleotides and selective killing of lymphocytes with relatively little toxicity in other tissues (2). The drug is used widely as a treatment for lymphoid malignancies and is especially effective in hairy cell leukemia (3,4). Because of positive results from our therapeutic trial of cladribine in progressive MS (5,6), we undertook this study to evaluate cladribine in patients with the relapsing-remitting form of MS.

Key words: immunosuppression; 2-chlorodeoxyadenosine.

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METHODS

Patient Selection

The study subjects were 52 patients with clinically definite relapsing-remitting MS for at least 1 year. All patients had a history of two or more relapses in the previous 2 years and Extended Disability Status Score (EDSS) scores of 6.5 or less at time of study entry. The majority of patients had been followed at Scripps Clinic. Exclusion criteria included: 1) prior treatment with an immunosuppressive drug within 3 months; 2) a serum creatinine of >1.5 mg/dl; 3) serum glutamic-oxaloacetic transaminase/serum glutamic-pyruvic transaminase or alkaline phosphatase elevated to twice the upper limit of normal; 4) baseline neutrophil counts of $<1600/\mu l$ or platelet counts of $<130{,}000/\mu l$; and 5) previous total lymphoid irradiation or prior extensive myelosuppressive chemotherapy.

The study plan, risks, and potential benefits were explained to each patient in detail. All patients gave informed consent to participate in the study.

Study Design

An 18-month, randomized, placebo-controlled, double-blind study was conducted in the facilities of the General Clinical Research Center (GCRC) of Scripps Clinic. After completion of screening evaluations, 52 patients were stratified according to gender, age (in 10-year intervals), and degree of disability as mea-

sured by Scripps Neurological Rating Scale (SNRS; 7) (in 10-point intervals). The stratified groups were then randomized in blocks of four to either the placebo arm or the cladribine arm. In all, 27 patients were randomized onto the cladribine arm and 25 onto the placebo arm. Throughout the study, patients, neurologists, nurses, and the neuroradiologist remained blinded to treatment assignment. A pharmacist was informed of patient assignment by code in order to dispense placebo or the appropriate dose of cladribine to each patient.

In all patients, clinical neurological exams plus SNRS and EDSS rating scales were performed at baseline and repeated by the same neurologist every month for the first year, every 3 months for the second year, and within 48 hr or less of report by a patient of a relapse. A clinical relapse was defined as the appearance of new symptoms or worsening of an existing symptom, attributable to MS and accompanied by objective worsening of neurological findings. To be scored as a relapse the alterations must have been preceded by disease stability or improvement lasting for at least 30 days, and the worsening must have lasted at least 24 hr and occur in the absence of fever. Relapse severity was rated as follows: 1) mild relapse—decrease in SNRS of 1–7; 2) moderate relapse —decrease in SNRS of 8–14; or 3) severe relapse—decrease in SNRS of 15 or greater.

Magnetic resonance imaging (MRI) of the brain was performed on a 1.5 T Signa scanner (General Electric, Milwaukee, WI) for each patient at baseline, and then monthly for the first year and every 6 months the second year. T1-weighted scans were obtained in the sagittal and axial planes. Axial scans of 3 mm thickness and zero interslice gaps were done about 10 min after the intravenous injection of gadopentetate dimeglumine (Magnevist, Berlex Laboratories). Special attention was given to careful repositioning of patients to guarantee reproducible slice positions. The regions of contrast enhancement on T1-weighted scans were outlined by hand on filmed images. All scans were interpreted and marked by the same neuroradiologist (J.Z.), who had no knowledge of patient treatment assignment. These were then duplicated by a technologist using the taped raw data and a computer workstation [ANALYZE (8), Rochester, MN]. Quantitation of MRI findings involved the determination of lesion areas on the consecutive sections of the T1-weighted scans as interpreted by one of two skilled technologists, then calculation of volumes by assuming homogeneity of lesions across the sections. Initially, the taped raw data from the individual scans were read into a volume-rendering software program, ANALYZE, running on a Hewlett-Packard 712/60 workstation. Our methodology for lesion area determination is a semiautomated quantitative technique adapted from Wicks et al. (9) and Filippi et al. (10).

Drug Administration

In contrast to our earlier study of intravenous cladribine in progressive MS (5), the drug was administered subcutaneously because of greater ease of administration and because it has now been established that the pharmacological properties and response rates of cladribine in lymphoproliferative diseases are the same if the drug is given either intravenously or subcutaneously (11). Each patient received a course of five consecutive daily subcutaneous injections of cladribine, 0.07 mg/kg/day or an equivalent volume of saline placebo, fractionated into two or three injection sites, and given monthly for 6 months for a total cumulative dose of 2.1 mg/kg of cladribine. A complete blood count was obtained before each monthly course of treatment and reviewed by the pharmacist, and the next dose of cladribine was given only if blood count safety criteria were met according to an algorithm designed for this purpose by one of us (E.B.; Table 1). If these criteria were not met, a placebo dose was substituted. The study design included eight monthly courses. The last two courses ordinarily consisted of placebo, but if a drug dose had been omitted because of blood count inadequacy, then active drug could be given at month 7 or 8 instead of placebo.

Statistical Considerations

Two primary outcome measures were identified: 1) the joint frequency and severity of clinical relapses as judged by neurological examination; and 2) the numbers of enhancing lesions on T1-weighted MRI brain

Table 1. Pretreatment safety criteria for monthly courses of cladribine in multiple sclerosis

1.	Platelet count must be:
	a. 200,000 or higher, or
	b. Between 150,000 and 200,000 and represent more than 50% of previous pretreatment platelet count, or
	c. Between 125,000 and 150,000 and represent at least 80% of previous pretreatment platelet count
2.	Absolute granulocyte count must be greater than 1000
3.	Hemoglobin level must not have declined:
	a. More than 1.5 g/dl from previous monthly pretreatment level, or
	b. 3 g/dl or more from baseline

scans. Outcomes were to be assessed at 1 year. A sample size of 25 patients per group would be sufficient to detect a decline in the annual rate of exacerbations, from 1 in the placebo group to 0.5 in the cladribine group, with a two-sided Poisson test at alpha level 0.05 (12). Similarly, on the basis of findings from our chronic progressive MS trial, we postulated that the frequency of enhancing lesions in the placebo group would remain at 50% throughout the course of this study, whereas the frequency of enhancing lesions in the cladribine-treated group would decline from 50% to <10% at 1 year. A sample size of 25 patients per treatment group would be sufficient to detect a difference of 50% versus 10% with a power of 0.90, using a two-sided binomial test at alpha level 0.05.

Our analyses were intent-to-treat, in that all data from every patient initially randomized either to placebo or to cladribine are included and reported for that initial treatment group. Blinded observations were undertaken up to 18 months from baseline (trial entry); hence, information is reported out to this period. We present primary analyses—that is, analyses of the primary outcomes at 1 year—and identify other analyses as secondary. We did not impute data values for any patient not observed out to 18 months.

Comparison of the joint frequency and severity of relapses between the two treatment groups was undertaken using Mantel's (13) extension of the Mantel-Haenszel procedure, here denoted Q_M. (Mantel's procedure can incorporate arbitrary scores for the degree of relapse, but we chose to score objectively by means of ranks based on drop in SNRS score, separately in each monthly summary table of relapses, as cross-classified by treatment group.) A stratified version of Mantel's test and a general linear model with Poisson link (that is, a Poisson regression model; 14) were also used to evaluate the significance of covariate information as predictors of clinical relapse. Confidence intervals for relapse rates were calculated under the assumption that the numbers of events followed a Poisson distribution in each treatment group. Comparison of the frequency of enhancing lesions on T1-weighted MRI scans over time was done with McNemar's test for paired data (within treatment groups) and Fisher exact test (between treatment groups); logistic regression was also used to assess the significance of covariate information. A nonparametric repeated measures analysis of variance procedure (15) was used to compare neurological performance scores (EDSS and SNRS) between the two treatment groups over the course of the study. The EDSS and SNRS scores were considered to be secondary outcome measures since little change might be expected between the two groups over the relatively short time frame of the study. Two-sided p values relative to the null distributions of the observed test statistics are reported.

Intrarater reliability of the determination of presence of enhancing lesions on T1-weighted MRI scans was assessed by means of a test-retest of 20 scans by the examining neuroradiologist (J.Z.). Discrepancies between the two independent evaluations were 15% (3/20). In a similar spirit, both examining neurologists (J.R. and J.S.) participated in a study of inter-rater and intrarater reliability, with regard to the neurological rating scales. Twenty patients (J.R., 10; J.S., 10) were assessed by the same examiner twice on the same day, the period between examinations ranging from 135 min to 240 min. Intrarater agreement for one examiner (J.S.) on the EDSS was perfect; the weighted κ coefficient of agreement (16) for the other examiner was 0.997. The weighted κ coefficients of agreement between the paired SNRS scores were 0.999 for both examiners. Separately, 20 patients were independently assessed by each examiner on the same day. Inter-rater agreement was high: the weighted κ coefficient of association was 0.990 for the EDSS and 0.957 for the SNRS. Inter-rater agreement on the EDSS was 100% for all sets of examinations when agreement was defined as a difference of less than or equal to 1.0, and 95% when agreement was defined as a difference of less than or equal to 0.5. Inter-rater agreement on the SNRS was 95% when agreement was defined as a difference of no more than 10 points, and 90% when agreement was defined as a difference of no more than 5 points.

RESULTS

Trial Considerations

There was one withdrawal on the placebo arm at 3 months (conversion disorder complicating assessment of underlying MS), and one withdrawal on the cladribine arm at 4 months (patient moved out of state); all of the remaining patients received standard intervention without deviations, as specified in the protocol. Thus, 26 cladribine patients and 24 placebo patients were available for evaluation at 12 months. During the period from 12 to 18 months, five patients on placebo withdrew: two patients moved out of state, two withdrew for unspecified reasons, and one withdrew because of worsening MS. One patient receiving cladribine also withdrew because of worsening MS. Thus, 25 cladribine patients and 19 placebo patients were available for evaluation the entire 18-month period. Figure 1 depicts the trial profile. During the period from 12 to 18 months, the blinding was removed from two cladribine patients and two placebo patients.

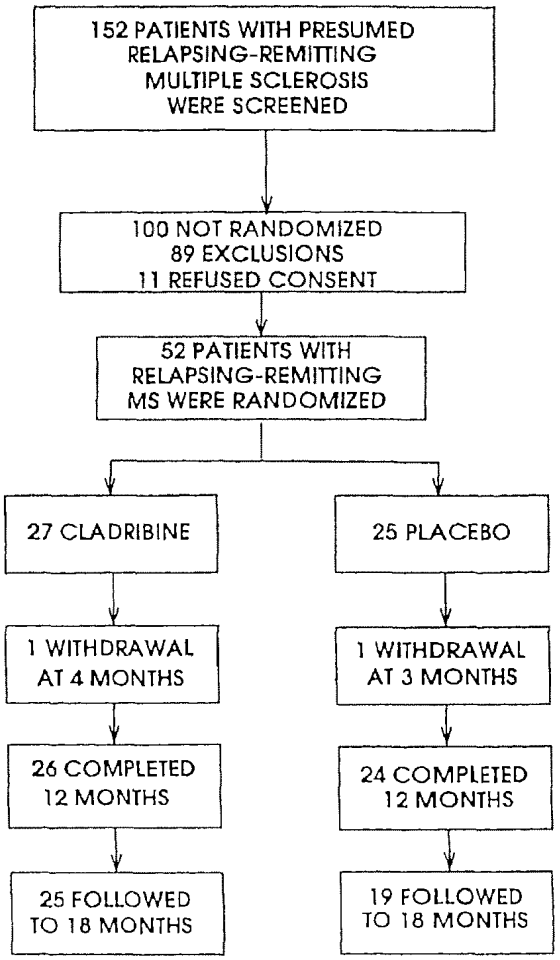

Figure 1. Trial profile of the cladribine relapsing-remitting MS clinical trial.

Because of potential bias, information from these patients concerning their frequency and severity of exacerbations subsequent to the point of unblinding is not used in the calculation and comparison of exacerbation rates between the two treatment groups.

Demographic and Baseline Characteristics in the Two Treatment Groups after Randomization

The two groups were similar in terms of baseline clinical characteristics (Table 2). Each group had an approximate 2:1 female-to-male preponderance and comparable mean age, disease duration, and baseline EDSS. Patients randomized to cladribine therapy averaged a slightly greater number of exacerbations in the 12 months prior to study entry than patients randomized to placebo.

Table 2. Baseline demographic and clinical characteristics

	Placebo (n = 25)	Cladribine (n = 27)
Sex		
Male	7	9
Female	18	18
Race		
White	25	24
Other	0	3
Age (years)		
Mean	39.8	43.4
25th percentile	36.5	38.5
50th percentile	41	44.5
75th percentile	44	49.5
Range	31–52	30–52
Years with symptoms		
Mean	9.1	10.2
25th percentile	3.5	4.5
50th percentile	9	8
75th percentile	12.5	12.5
Range	1–25	1–29
Number of exacerbations in previous year		
1	13	5
2	5	16
3 or 4	7	6
Baseline EDSS		
Mean	3.8	3.9
25th percentile	2.5	2.3
50th percentile	3.5	5.5
75th percentile	5.3	5.5
Range	2–6.5	2–6.5
Baseline SNRS		
Mean	75.8	76.1
25th percentile	67	66
50th percentile	75.5	78.5
75th percentile	86	86.5
Range	54–98	41–93

EDSS, Extended Disability Status Score. SNRS, Scripps Neurological Rating Scale.

Effect of Cladribine on Outcome Measures

Figure 2 depicts the frequency and severity of exacerbations for all patients enrolled in the study. We examined the joint distribution of frequency and severity over months 7 through 12 for treatment comparisons: on the basis of our prior experience (17), we expected the maximum immunosuppression on cladribine therapy would not be achieved prior to month 7. Using the extended Mantel-Haenszel procedure, we found that there is a statistically significant reduction in the frequency and severity of exacerbations in the cladribine group compared to the placebo group over months 7 through 12 ($Q_M = 2.30$, $2p = .021$). Over this period, the relapse rate in the cladribine group

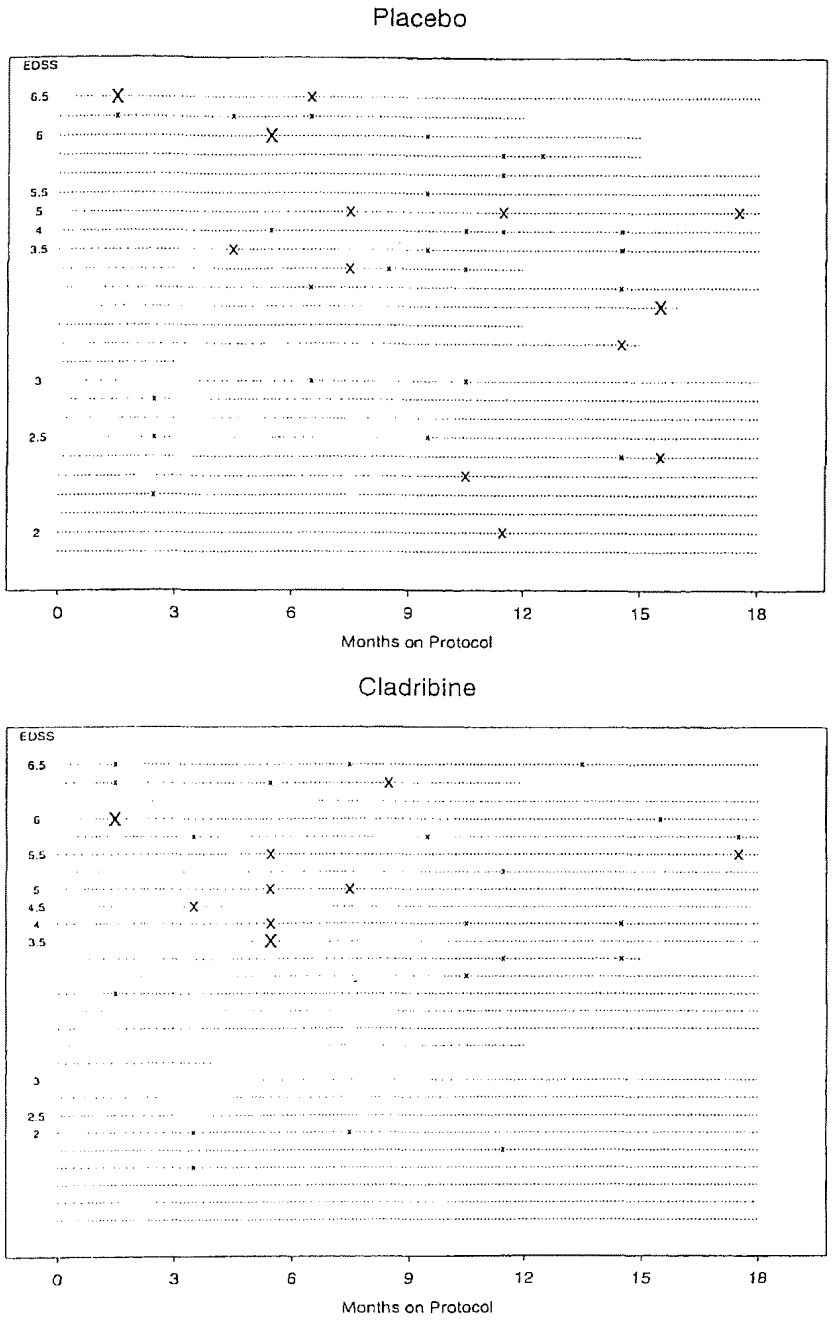

Figure 2. Event charts for frequency and severity of exacerbations over the course of the study in placebo-treated and cladribine-treated patients.
Within treatment groups, patients are ordered in terms of decreasing EDSS scores at baseline; patients with identical EDSS scores at baseline are ordered by numbers of new exacerbations. Mild, moderate, or severe exacerbations are denoted by progressively heavier X's.

was 0.77 per year [95% confidence interval (CI), 0.37–1.41], compared to 1.67 per year in the placebo group (95% CI, 1.02–2.57). With Poisson regression, we identified treatment along with two other covariates, baseline EDSS and number of exacerbations in the year prior to start of treatment, as significant predictors of relapse over months 7 through 12 (with fewer relapses being associated with cladribine therapy, lower EDSS scores at baseline, and fewer exacerbations in the year prior to start of treatment). In secondary analyses, we found that the reduction in the distribution of frequency and severity of exacerbations in the cladribine group relative to the placebo group is sustained at 18 months: $Q_M = 2.59$, $2p = .010$ over

the 1-year period from month 7 through month 18. Over this extended period, the exacerbation rate in the cladribine group was 0.66 per year (95% CI, 0.37–1.05) compared to 1.34 per year in the placebo group (95% CI, 0.90–1.93).

Figure 3 compares the frequency and severity of relapses at 6-month intervals during the study with the frequency of relapses in the 12 months preceding treatment with drug or placebo. It is apparent that there was striking improvement in the first 6 months of the study, when injections of placebo or cladribine were being given 5 days of each month, regardless of whether the patients received placebo or active drug. When injections were stopped, the frequency of relapses in the placebo group returned to its baseline, pretreatment frequency, but the frequency and severity of relapses continued to decline in the patients who received cladribine.

MRI results (Fig. 4, Table 3) revealed complete suppression of enhancing lesions after study month 6 in the cladribine group, whereas lesion enhancement persisted in the placebo group. Formally, with regard to the primary outcome measure at 12 months, there is a highly significant decrease in the occurrence of enhancing lesions at 12 months relative to baseline in the cladribine group ($2p < .0003$ by McNemar's test). In contrast, there is a slight increase in the occurrence of enhancing lesions at 12 months relative to baseline in the placebo group ($2p = .109$ by McNemar's test); and, the frequency of enhancing lesions is significantly greater at 12 months in the placebo group than in the cladribine group ($2p = .0001$ by Fisher exact test). In secondary analyses, we find a significant reduction in the frequency of enhancing lesions experienced by the cladribine group relative to baseline already at month 7 and persisting at month 18 ($2p < .0005$ by McNemar's test at each time point). Moreover, the frequency of enhancing lesions in the placebo group is already significantly greater than that in the cladribine group by 7 months ($2p = .0001$ by Fisher) and remains so at 18 months ($2p = .002$ by Fisher). No significant predictors of enhancing lesion presence other than treatment were found by logistic regression.

We found no significant differences between the treatment groups in either EDSS or SNRS scores, the secondary outcome parameters, over 18 months ($p > .5$ for each; Fig. 5).

Adverse Events and Side Effects

Infections were limited to an episode of mild segmental herpes zoster that occurred in two cladribine-

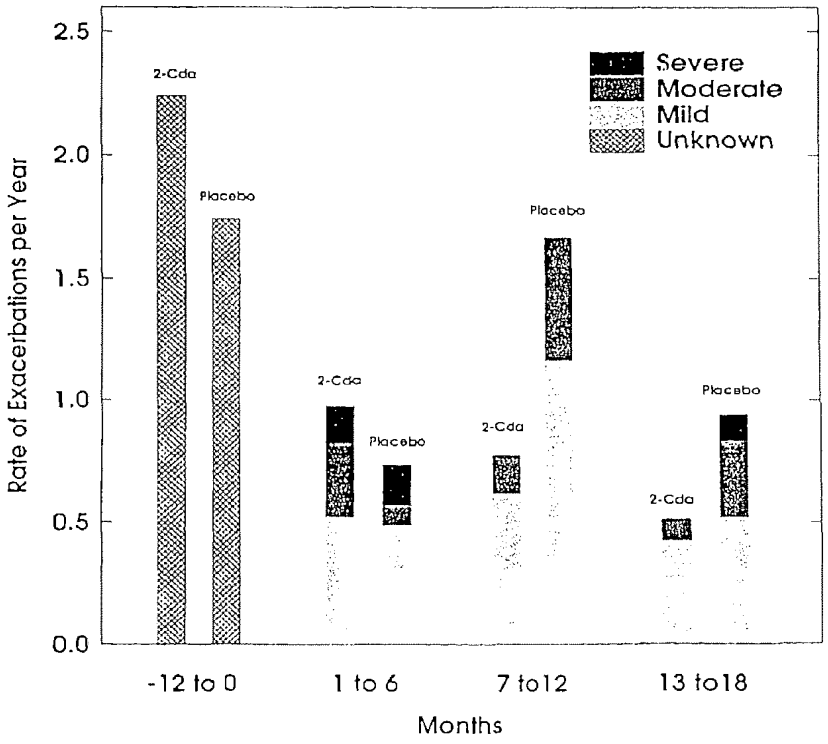

Figure 3. Rates of exacerbations for the two treatment groups.
The number of exacerbations for the year prior to initiation of treatment was obtained from the patient's history. Over the course of the trial itself, exacerbations were documented by the neurologist and classified as mild, moderate, or severe as explained in the text. For purposes of comparison, rates are presented as relapses per year.

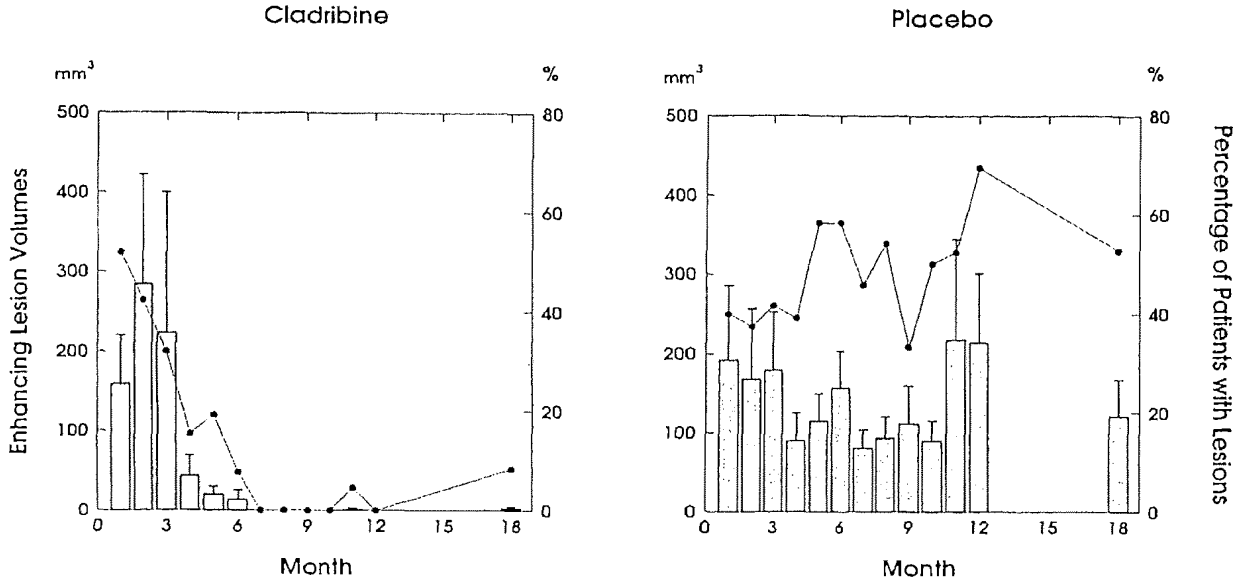

Figure 4. Average volumes of enhancing lesions on T1-weighted scans (bars, left vertical axis), and proportions of patients with enhancing lesions (dots, right vertical axis), for the two treatment groups over the course of the trial.
Averages and proportions were computed on the basis of all available MRI data at each time point. Standard error bars are also given for the MRI volumes.

treated patients and in one patient receiving placebo. Acyclovir was administered orally in each case. Cladribine-treated patients experienced no side effects that might have led to unblinding of patients or examining neurologists. Average lymphocyte counts in the cladribine group declined as expected to a nadir at 7 months of 0.4 × $10^3/\mu l$. The average platelet count in the cladribine group declined modestly to a low of >200 × $10^3/\mu l$ at 6 months, and the average hemoglobin to a low of >13.5 g/dl at 11 months, but there were no individual cases of significant thrombocytopenia, anemia, granulocytopenia, or generalized marrow suppression (Fig. 6).

Table 3. Presence of enhancing lesions by MRI over the course of the trial

Time	Placebo		Cladribine	
	Lesions absent	Lesions present	Lesions absent	Lesions present
Baseline	15	10	13	14
7 months	13	11	25	0
12 months	7	16	25	0
18 months	9	10	22	2

Note: One cladribine patient did not have an MRI during month 7, one placebo patient and one cladribine patient did not have an MRI during month 12, and one cladribine patient did not have an MRI at month 18.

DISCUSSION

In view of the long-lasting lymphopenia and relatively low toxicity from cladribine, we began studies in 1990 with progressive MS patients in the hope that depletion of immunocytes might be beneficial. Because initial observations were encouraging, we undertook a 2-year, placebo-controlled, double-blind study in 51 patients in which cladribine (total dose = 2.8 mg/kg) or placebo was administered during the first year via a central venous access device using a portable infusion pump. A favorable effect on neurological performance scores and on MRI findings was documented in patients treated with cladribine (5,6).

A multicenter trial has subsequently been conducted in 159 patients with progressive MS using cladribine subcutaneously at a total dose of 2.1 mg/kg. Unexpectedly, there was no worsening of neurological performance scores in the placebo arm and therefore no significant differences were seen between cladribine and placebo-treated groups. This study was probably underpowered due to inclusion of more patients with advanced disability (18). However, marked suppression of enhancing MRI lesions with cladribine treatment was confirmed in the pro-

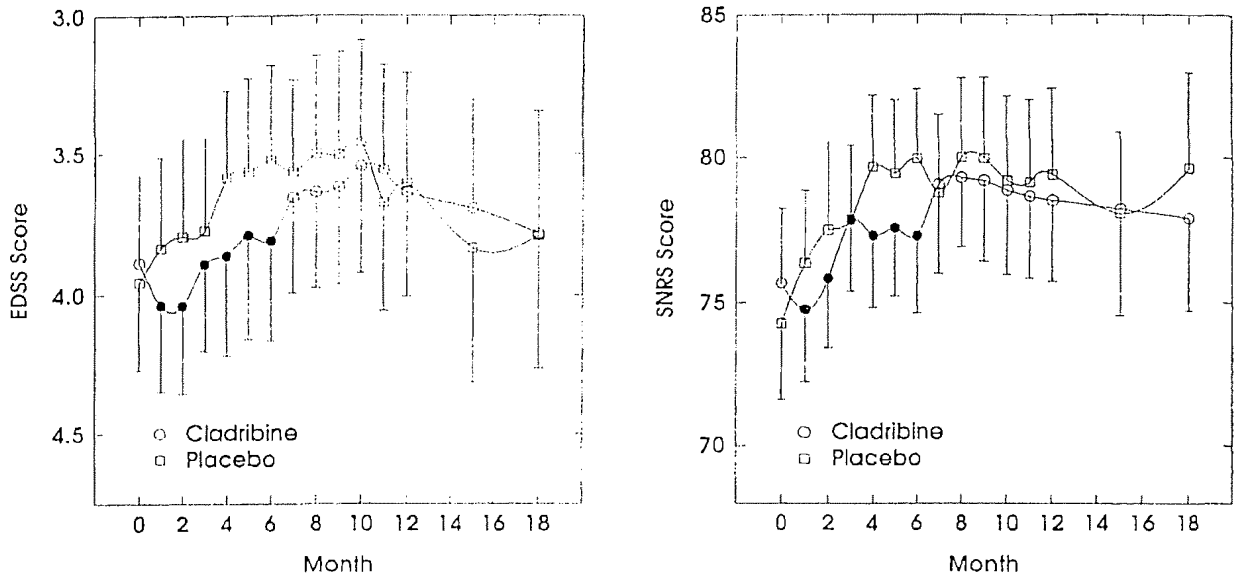

Figure 5. Changes in the Kurtzke (EDSS) and Scripps (SNRS) rating scores.
Solid symbols indicate when cladribine was administered. Means and pointwise standard error bars are shown.

gressive MS multicenter study and no significant toxicity was observed.

The results in relapsing-remitting MS, as reported in the current study, indicate that cladribine given subcutaneously at a total dosage of 2.1 mg/kg appears to be safe and effective in reducing the rate and severity of clinical exacerbations for at least the relatively short duration of the study. Particularly striking was the placebo effect noted in the first 6 months of our study, when patients were receiving injections; the relapse frequency dropped to about one half in both groups of patients. In the second 6 months, however, when no injections were given but after immunosuppression had been achieved in the drug-treated group, the relapse frequency returned to baseline in patients who had received placebo, but patients who had received drug continued to enjoy relative freedom from exacerbations, an effect that continued into the second year of the study.

Our primary analyses are predicated on outcomes at 1 year following randomization. During this period, all patients received the treatment to which they had been randomized; that is, treatment allocation and treatment actually received were identical for everyone. Two patients, one randomized to placebo and the other to cladribine, withdrew from the study during the first year. Hence, the attrition rate was <5% on each arm over the formal length of the trial. The results of our primary analyses at 1 year, comparing the joint frequency and severity of exacerbations between the treatment groups, and comparing the frequencies of enhancing lesions, are insensitive to the loss of information from these patients. With regard to the second primary end point, the presence of enhancing lesions of T1-weighted scans, it is clear from Table 3 that even under the least favorable scenario for cladribine—no enhancing lesions in the placebo withdrawal, but enhancing lesions in the cladribine withdrawal—the favorable outcome of cladribine therapy relative to placebo at 1 year would remain overwhelmingly significant. The lack of any demonstrable difference in neurological disability (EDSS and SNRS scores) between the cladribine and placebo groups is of uncertain significance since neither treatment group, including the placebo group, showed significant worsening over the duration of the study.

The almost complete suppression of MRI-enhancing lesions with cladribine is a very robust treatment effect similar to that previously reported with cladribine in progressive MS (5,6,18) and exceeding the effect of interferon beta-1a (19). Since enhancement of MRI lesions is thought to reflect active disease (20), the question arises as to how some cladribine-treated patients continued to have clinical relapses, but with no apparent lesion enhancement on serial MRI scans.

The mechanism of action of the beneficial effect of cladribine on MS is presumably related to the selective and sustained depletion of lymphocytes. In a previous study of cladribine in chronic progressive

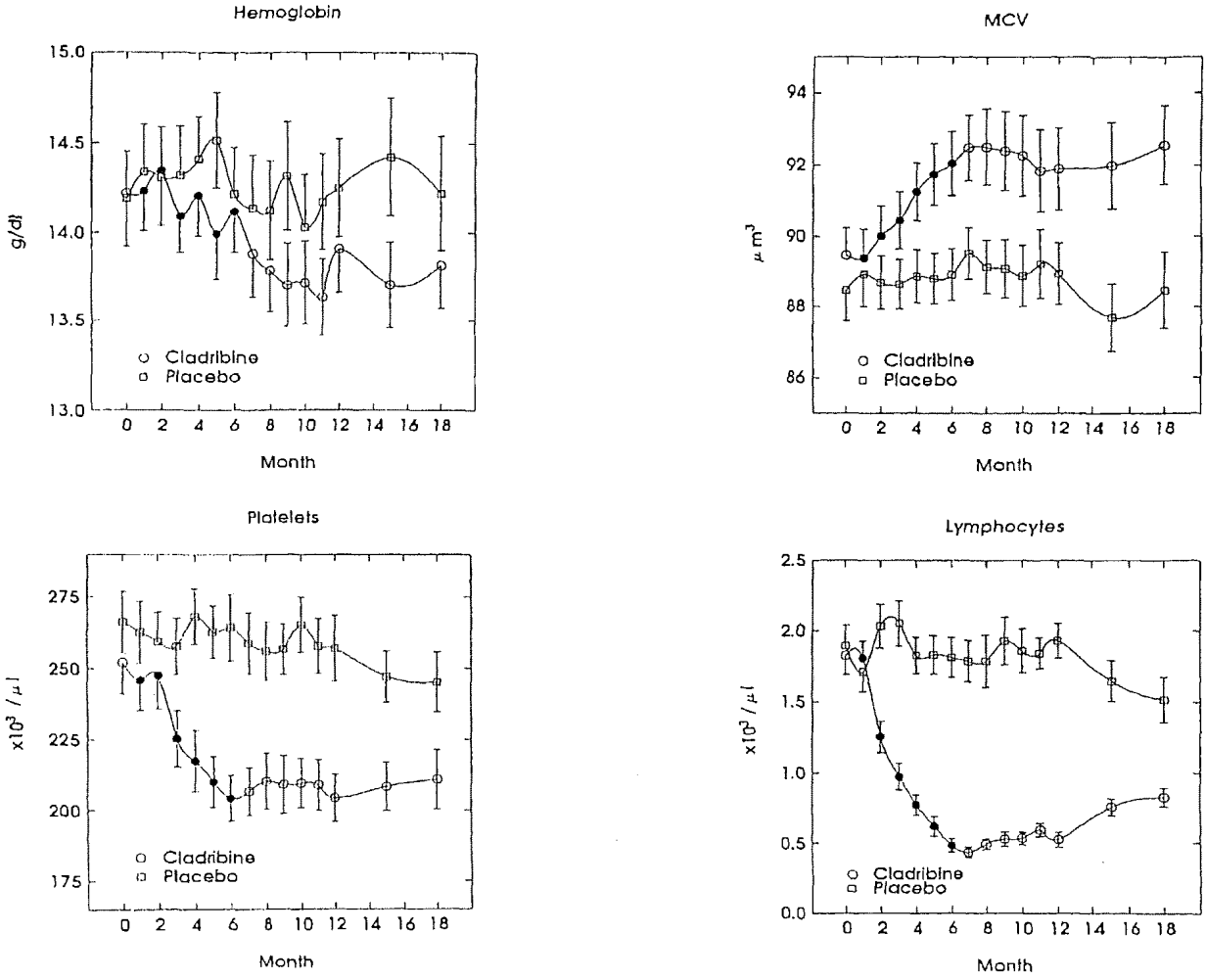

Figure 6. Serial blood cell counts over the course of the trial.
Solid symbols indicate when drug was administered. Means and standard error bars are shown.

MS (6), there was an approximately four-fold decrease in the CD4/CD8 "helper/suppressor" ratio and an approximately five-fold reduction of CD25-activated T and B lymphocytes, which lasted for many months after a 4-month course of the drug. By comparison, treatment of MS with the general immunosuppressive drugs cyclophosphamide (21) or chlorambucil (22) produced a relatively transient two-fold decline in the CD4/CD8 ratio. This difference suggests that cladribine may have a more potent immunosuppressive effect in MS than other drugs.

The duration of the beneficial effect on relapsing MS after a course of cladribine could not be determined from this study since the patients were unblinded after 18 months. However, an estimate based on observations of cladribine in chronic progressive MS (6) would indicate that beneficial effect should eventually decline and would not be expected to last much beyond 2 years. The lengthy but impermanent duration of effect of cladribine means that retreatment will be necessary if cladribine is to become a practical long-term therapy for MS. At present, there are few data on the safety of long-term retreatment with cladribine in a nonmalignant disease such as MS. Twenty-four patients from our initial study of cladribine in chronic progressive MS have been retreated (0.07 mg/kg × 5 days × 4 courses) because of a recurrence of disease worsening. There were no instances of marrow suppression or thrombocytopenia in these patients, but 6 (25%) developed herpes zoster with a second course of cladribine as compared to 2 of 51 (4%) with the first treatment. Repeated treatment

raises long-term toxicity issues including: 1) susceptibility to opportunistic infection; 2) marrow stem cell depletion; and 3) an increase in the incidence of malignancy, known to occur in association with other long-term immunosuppressive treatments (23).

Since currently available treatments for relapsing MS are only partially effective and because, in the case of interferons, there is concern that the beneficial effect may be lost due to induction of neutralizing antibodies (24), new treatments need to be developed. In this regard, cladribine shows promise as a relatively safe and at least temporarily effective treatment in reducing the frequency and severity of MS exacerbations and in suppressing new contrast-enhancing MRI lesions.

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REFERENCES

1. Carson D.A., Kaye J., Matsumoto S., et al. Biochemical basis for the enhanced toxicity of deoxyribonucleosides toward malignant human T cell lines. *Proc. Natl. Acad. Sci. U.S.A.* 76: 2430–2433, 1979.
2. Carson D.A., Wasson D.B., Taetle R., and Yu A. Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. *Blood* 62: 737–743, 1983.
3. Carson D.A., Wasson D.B., and Beutler E. Antileukemic and immunosuppressive activity of 2-chloro-2'-deoxyadenosine. *Proc. Natl. Acad. Sci. U.S.A.* 81: 2232–2236, 1984.
4. Beutler E. Cladribine (2-chlorodeoxyadenosine). *Lancet* 340: 952–956, 1992.
5. Sipe J.C., Romine J.S., Koziol J.A., et al. Cladribine in treatment of chronic progressive multiple sclerosis. *Lancet* 344: 9–13, 1994.
6. Beutler E., Sipe J.C., Romine J.S., et al. The treatment of chronic progressive multiple sclerosis with cladribine. *Proc. Natl. Acad. Sci. U.S.A.* 93: 1716–1720, 1996.
7. Sipe J.C., Knobler R.L., Braheny S.L., et al. A neurologic rating scale (NRS) for use in multiple sclerosis. *Neurology* 34: 1368–1372, 1984.
8. Biomedical Imaging Resource: Mayo Foundation. *Analyze reference manual version 7.0.* West Sussex, U.K.: CNSoftware Ltd., 1993.
9. Wicks D.A.G., Tofts P.S., Miller D.H., et al. Volume measurement of multiple sclerosis lesions with magnetic resonance images. *Neuroradiology* 34: 475–479, 1992.
10. Fillipi M., Paty D.W., Kappos L., et al. Correlations between changes in disability and T2-weighted brain MRI activity in multiple sclerosis: A follow-up study. *Neurology* 45: 255–260, 1995.
11. Liliemark J., Albertioni F., Hassan M., et al. On the bioavailability of oral and subcutaneous 2-chloro-2'-deoxyadenosine in humans: Alternative routes of administration. *J. Clin. Oncol.* 10: 1514–1518, 1992.
12. Cox D.R. Some simple approximate tests for Poisson variates. *Biometrics* 30: 354–360, 1953.
13. Cohen J. Weighted kappa: Nominal scale agreement with provision for scaled disagreement or partial credit. *Psychol. Bull.* 70: 213–220, 1968.
14. Mantel N. Chi-square tests with one degree of freedom: Extensions of the Mantel-Haenszel procedure. *J. Am. Statist. Assoc.* 58: 690–700, 1963.
15. McCullagh P. and Nelder J.A. *Generalized linear models.* London: Chapman and Hall, 1989.
16. Koziol J.A., Maxwell D.A., Fukushimia M., et al. A distribution-free test for tumor growth curve analyses with application to an animal tumor immunotherapy experiment. *Biometrics* 37: 383–390, 1981.
17. Beutler E., Koziol J., McMillan R., et al. Marrow suppression produced by repeated doses of cladribine. *Acta Haematol.* 91: 10–15, 1994.
18. Rice G.P.A. and the Cladribine Study Group: Cladribine and chronic progressive multiple sclerosis: The results of a multicenter trial [abstract]. *Neurology* 48: 1730, 1997.
19. Jacobs L.D., Cookfair D.L., Rudick R.A., et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. *Ann. Neurol.* 39: 285–294, 1996.
20. Oger J., Kostrukoff L.F., Li D.K.B., and Paty D.W. Multiple sclerosis: In relapsing patients immune functions vary with disease activity as assessed by MRI. *Neurology* 38: 1739–1744, 1988.
21. Moody D.J., Fahey J.L., Grable E., et al. Administration of monthly pulses of cyclophosphamide in multiple sclerosis patients. *J. Neuroimmunol.* 14: 175–182, 1987.
22. Chiapelli F., Myers L., Ellison G.W., et al. Preferential reductions in lymphocyte subpopulations induced by monthly pulses of chlorambucil: Studies in patients with chronic progressive multiple sclerosis. *Int. J. Immunopharmacol.* 13: 455–461, 1991.
23. Penn I. Tumors arising in organ transplant recipients. *Adv. Cancer Res.* 28: 31–61, 1978.
24. Paty D., Goodkin D., Thompson A., and Rice G. Guidelines for physicians with patients on IFNβ-1b: The use of an assay for neutralizing antibiotics (NAB). *Neurology* 47: 865–866, 1996.

(14)

PROBLEMS OF EXPERIMENTAL TRIALS OF THERAPY IN MULTIPLE SCLEROSIS: REPORT BY THE PANEL ON THE EVALUATION OF EXPERIMENTAL TRIALS OF THERAPY IN MULTIPLE SCLEROSIS

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Introduction

Multiple sclerosis has become a focus of growing interest to neurologists, general physicians, and the public. The disorder has an uneven but worldwide distribution and is present in approximately 30 to 60 people per 100,000 in the temperate zones of the world. Though some of these individuals remain unaffected by serious disability, in the majority the disease leads to crippling and ultimately premature death. To the public a special appeal lies in the frequent occurrence of multiple sclerosis in the young adult, often with disruption of early family life.

To date no satisfactory treatment for the amelioration or cure of the disease has been discovered. Since its etiology and pathogenesis have eluded detection, it is not surprising that therapeutic attempts have been empiric and often unscientific. Reports of benefit from various types of therapy based on uncontrolled observations have led to unwarranted and occasionally widespread application of methods whose value remained unsubstantiated. In recent years criticisms have been made of the lack of adequate controls in trials of therapy. The difficulties inherent in judging the effects of therapy have been stressed.[1, 2] These are: (1) Lack of precision in diagnosis. (2) The erratic and unpredictable course of the disease with periods of spontaneous remission of symptoms. (3) Lack of a direct method for investigating activity of the disease. (4) The existence of only crude parameters for quantitating and recording the clinical course of the disease. (5) The irreversibility of gliosis and its masking effect on disease activity elsewhere in the nervous system. (6) Psychological disturbances, including hysterical tendencies, in some patients. (7) Problems of keeping large groups of patients under standard conditions of therapy or control for long periods (necessary because of the chronicity and erratic nature of the disease). Attempts to apply some degree of scientific control to therapeutic investigations in multiple sclerosis, however, have been made recently.[3–6]

Because of the prevalence of poorly controlled therapy reports, the National Institute of Neurological Diseases and Blindness provided financial support for a Symposium on the Evaluation of Drug Therapy in Neurologic and Sensory Diseases* which was held at the University of Wisconsin in May, 1960. Ten panels composed of investigators, clinicians, and statisticians met to consider the problem of experimental trials of therapy in various areas of nervous system disease. The results of the discussions were subsequently published.[6] The conclusions reached by the Panel on Multiple Sclerosis comprised essentially a statement of the problems that needed study and solution to permit the design of a protocol for sound experimental trials of therapy. Thus, they formed merely a point of departure for future study.

As an outgrowth of the initial considerations of the Panel on Multiple Sclerosis, a continuing panel was formed which held a series of workshops on the problem.

*Chairman, Francis M. Forster.

An early aim was to achieve definition and uniformity in the use of terms in order to provide a common ground of acceptable terminology among different investigators and writers. The overall aim was to relate established principles of scientific investigation and control[7-10] to experimental trials of therapy of multiple sclerosis. A need was believed to exist for a statement of criteria to provide guide-lines for potential investigators and for editors of scientific journals. The conclusions and recommendations derived from the work of the group are embodied in this report.*

It was realized that all the problems inherent in future experimental trials of therapy in multiple sclerosis would not be detected in this preliminary study and undoubtedly many would come to light only as trials of therapy actually were undertaken. Also recognized was the fact that scientific evaluation of therapy might require relatively large numbers of patients followed over a reasonable length of time, thus necessitating a cooperative approach on the part of investigators representing several institutions. This fact enhanced the importance of a carefully devised protocol with standardization of observational techniques. Though the study sought to delineate the requirements of controlled trials of therapy, the report does not presume to outline a specific protocol; rather the problems of control are taken up, attempts made to clarify them, and recommendations made.

The principles originally stated by Fisher[7] necessary to insure validity and reliability in therapeutic experiments are those of replication, randomization, and comparison. The later sections of this report have as their aim the clarification of methods of achieving these principles.

I. The Experimental Subjects

Definition of Multiple Sclerosis

Multiple sclerosis is a disorder characterized in cross-section by symptoms and signs of neurologic dysfunction indicating multiple and separate lesions in the central nervous system. Symptoms appear longitudinally in the form of acutely or slowly developing episodes scattered over a period of time. Individual attacks may assume a variety of patterns. The overall course is made up of multiple attacks or of erratic or steady progression over prolonged periods, usually many years.

A common pattern of attack is one of sudden or gradual development of neurologic dysfunction followed by gradual, occasionally rapid disappearance of symptoms. A second common pattern is one of sudden or gradual onset with progression of disability to a particular level at which symptoms or signs remain with minor fluctuations for an indefinite period. A third pattern is one of sudden or gradual onset of symptoms and signs which reach a peak, and then gradually subside, but not completely, leaving the patient with some degree of residual dysfunction. Regardless of the course assumed by an attack, subsequent recurrence or steady progression usually leads ultimately to chronic and permanent disability. Rarely, a fulminating attack exhibits rapid onset of neurologic dysfunction with progression over a short period to death.

Diagnostic Criteria

Clinical diagnostic criteria. The diagnosis in most cases, even when termed "clinically definite" must remain one merely of high probability because of the lack of specific diagnostic tests. The familiar list of common symptoms and signs of the

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disease is of limited value since these may be caused by other diseases or be absent in multiple sclerosis. As emphasized below, laboratory tests may lend support to the diagnosis or aid in ruling out other conditions but, for the present at least, are not of use in confirming the diagnosis.

The following six criteria are deemed essential to characterize the disease state as clinically "definite multiple sclerosis."

a. There must be objective abnormalities on neurologic examination attributable to dysfunction of the central nervous system. Symptoms alone, no matter how suggestive, cannot be accepted as diagnostic of multiple sclerosis.

b. On neurologic examination or by history there must be evidence of involvement of two or more separate parts of the central nervous system.
(In determining multiplicity of lesions, certain signs or combination of them require cautious interpretation. For example, paleness of the temporal half of the optic disc is pathologic only if beyond the average range and if supported by evidence of impairment of visual acuity and/or fields or by a history suggesting a previous attack of retrobulbar neuropathy. It must also be kept in mind that "multiplicity of structural involvement" may occur in other central nervous system disease without fulfilling the requirements of "separateness," "multiplicity," or "dissemination" of lesions. Thus, if the involvement of several structures can be attributed to a single lesion at one locus, as is the case, for example, in tumor or infarct of the brainstem or compression of the spinal cord, such involvement cannot be considered as fulfilling the criterion of "multiplicity." Further, evidence reflecting the simultaneous and symmetric involvement of the lateral and posterior columns of the spinal cord, found commonly in disease of the central nervous system other than multiple sclerosis, cannot be interpreted as caused by multiple sclerosis in the absence of additional sites of involvement.)

c. The objective neurologic evidence of central nervous system disease must reflect predominantly white matter involvement, i.e., fiber tract damage. Thus, signs must consist mainly of optic nerve, cerebral subcortical, corticobulbar, corticospinal, medial longitudinal fasciculus, cerebellar subcortical, spinocerebellar, and long sensory tract (especially posterior column) dysfunction. More than a minor proportion of signs of lower motor neurone (brainstem, spinal nuclear gray matter, or peripheral nerve) dysfunction will disqualify a subject as having multiple sclerosis for purposes of an experimental trial of therapy.

d. The involvement of the neuraxis must have occurred temporally in one or the other of the following patterns:
(1) In two or more episodes of worsening, separated by a period of one month or more, each episode lasting at least 24 hours.
(2) Slow or step-wise progression of signs and symptoms, over a period of at least six months.

These arbitrary time-limits are necessary to exclude: (1) fluctuating or transitory neurologic impairment due to other causes (e.g., vascular); and (2) acute disseminated neurologic disease which is short-lived and nonrecurrent (such as encephalomyelitis).

e. The ages of the patient at the onset of the disease must fall within the range of 10 to 50 years, inclusive.
f. The patient's signs and symptoms cannot be explained better by some other disease process, a decision which must be made by a physician competent in clinical neurology.

It is realized there will be patients who do not meet precisely these criteria, yet have multiple sclerosis; conversely, there will be those who technically fit these criteria, yet will not be considered to have multiple sclerosis by the clinician. Nevertheless, all these criteria should be met for inclusion of patients in a test series.

Laboratory diagnostic criteria. No laboratory test pathognomonic of multiple sclerosis useful in the selection of cases for an experimental study has been discovered.

Minimum routine laboratory investigations. Patients accepted for an experimental trial of therapy should have the usual routine laboratory tests required in a thorough medical evaluation including a spinal puncture. These procedures assist in the differential diagnosis by providing negative evidence helpful in ruling out other conditions, as well as (in the case of the routine cerebrospinal fluid examination) revealing the occasional mild abnormalities consistent with the diagnosis of multiple sclerosis. The recommended tests are the following:

(1) Peripheral blood
    a. Hematocrit or hemoglobin.
    b. Total and differential leucocyte count.
(2) Urinalysis
(3) Blood serologic reaction for syphilis
(4) Fasting blood sugar
(5) Chest X-ray
(6) Spinal tap
    a. Manometrics
    b. Gross appearance
    c. Total and differential cell count
    d. Total protein content
    e. Serologic reaction for syphilis
    f. Colloidal gold curve or other modification of the Lange procedure.

Test lending support to the diagnosis of multiple sclerosis. Gamma-globulin content of cerebrospinal fluid. Because of the higher incidence, and higher levels, in this disease than in most other neurologic disorders (except neurosyphilis) of raised cerebrospinal fluid gamma-globulin content[11a, b, c, d] it is recommended that this be measured, preferably by the immunochemical method.[11a, e] In the absence of facilities for this technique, paper electrophoresis or even the relatively less accurate Zn SO_4 precipitation procedure may be utilized since significant correlations between the diagnosis of multiple sclerosis and pathologic elevations of this protein fraction have been shown in large series of cases investigated by these methods.[11b, c, d] On the basis of recent work, however, it seems likely that quantitative immunochemical measurements specifically of the gamma-2 (7s) subfraction of gamma-globulin will have the greatest diagnostic import.[11a, f]

Other tests. Further laboratory procedures may be required if the history, examination, or the above laboratory studies suggest the possibility of a disease in addition to, or other than, multiple sclerosis. Examples of such tests, including tests of potential importance for differential diagnosis of multiple sclerosis are shown in the following list:

(1) Blood urea nitrogen
(2) Glucose tolerance test
(3) Blood preparations for "L.E." cells
(4) Serum electrophoresis
(5) Gastric analysis and/or Schilling test
(6) Roentgenography of the skull or spine
(7) Myelography
(8) Electroencephalography
(9) Neostigmine diagnostic test

Criteria for Exclusion

Not all patients who have multiple sclerosis are suitable for inclusion in an experimental trial of therapy. Indiscriminate utilization of all subjects with a diagnosis of multiple sclerosis might open the way to the operation of factors which would introduce bias, or include subjects in whom testing was not feasible, or include some who might be detrimentally affected. Categories of cases unacceptable for a controlled therapeutic trial are defined under the following headings:

Advanced disease. A patient with "advanced multiple sclerosis" is one in whom both of the conditions listed below have been present for a period of one year or longer without definite functional improvement. (Definite functional improvement does not include such changes as fluctuations and transitory improvement in the degree of spasticity, subjective improvement unaccompanied by objective findings on neurologic examination, or improvement lasting less than 48 hours.)

a. Paraplegia or sufficient weakness of both legs to prevent ambulation. Patients able to walk with the aid of canes, braces, or the arm support of one individual, however, are not included in this category.
b. Sufficient ataxia, sensory loss, or weakness in both upper extremities to prevent independent feeding and dressing.

It is likely that in subjects with extensive and permanent destruction of parenchyma such tissue would be insusceptible of improvement from any form of therapy and that the clinical signs of both increased or decreased activity of the disease in other areas would be masked.

Dementia. While it does not seem feasible to define rigidly the degree of disqualifying mental impairment, loss of intellectual functioning sufficient to impair understanding and cooperation in the treatment and evaluation program should be cause for exclusion of the patient.

Concomitant disease. Subjects with other disorders, confusing or mimicking the picture of multiple sclerosis, or rendering evaluation unfeasible, impractical or harmful, should be excluded. These groups are as follows:

a. Other primary nervous system diseases productive of neurologic deficit (such as brain or spinal cord tumors, neuropathies, myopathies, symptomatic cranial or spinal traumas, neurosyphilis, and others).
b. Diseases with possible secondary effects on the nervous system (such as collagen diseases, hematologic disorders, embolic disease, advanced vascular disease, diabetes and others).

c. Diseases which limit function and therefore interfere with evaluation of the neurologic state (such as symptomatic pulmonary or cardiac disease, major amputations, ankyloses, or arthropathies).
d. Diseases so grave as to reduce life-expectation to less than the likely duration of the experimental trial of therapy (such as metastatic cancer).
e. Diseases for which the proposed therapy against multiple sclerosis is contraindicated (such as tuberculosis if steroids are to be used).

Observation of Course of Disease

Methods of Assessment of Neurologic Status

Assessment of status at fixed periods (serial neurologic examinations). a. *Method of recording and numerically scoring data from routine neurologic examination.* Though crude, the only available indicator of change in the disease process is change in peripheral function as measured heretofore by the standard neurologic examination. This may be supplemented by more detailed tests of mentation, visual acuity, visual fields, and bladder responsiveness (cystometrograms) which are refinements of bedside neurologic testing. Although such measures are neither wholly reliable nor accurate indicators of the amount or degree of underlying disease activity, more precise methods have not been available. It is desirable to bring together such neurologic data and the examiner's inferences into a more manageable form for recording and analysis. For the statistical analysis of results which ultimately will be a part of any program of evaluating therapy, the neurologic findings and related observations must be coded. To date, among the systems available for coding neurologic findings,[3, 4, 14-16] the one of Kurtzke[16] seems the simplest and least subject to error.

The method consists of grading the results of the neurologic examination and recording the scores in two complementary parts, (1) a set of grades expressing the degree of deviation from normal for each of eight categories of neurologic function, and (2) a general disability status score determined from an overall estimate of the patient's functional capacity. Only verifiable defects as elicited on neurologic examination are considered; symptoms per se are not included in the scoring process.

The separate categories of nervous system function are graded in terms of degree of dysfunction from 0 to 5 (or 6). These are: (1) mental functions, (2) visual functions, (3) brainstem functions, (4) cerebellar system functions, (5) pyramidal tract functions (6) sensory functions, (7) excretory functions, and (8) other functions. The disability score, estimated from the overall impairment of functional capacity, is based on a 0 to 10 point scale. Careful definitions are provided for the five to six grades of neurologic dysfunction in each category and of the 10 degrees of overall disability permitting the assignment of numerical scores.

The eight classes of neurologic function selected for scoring are representative of roughly separate anatomic quanta of central nervous system tissue in which the lesions of multiple sclerosis commonly occur. The method avoids the overlapping and indiscriminate duplication of scores found in other methods of numerical conversion that have been recommended. The dysfunction scores for the eight roughly separate anatomic-physiologic areas of the central nervous system cannot be logically added to give a valid numerical score of total dysfunction for reasons stated elsewhere.[16] They lend themselves, however, to following the progress

of separate dysfunctions (and hence, presumptively, that of lesions underlying them) during the course of the disease and in association with therapy. The disability score does give a crude quantitative picture of the progress of the disease as a whole and is utilized as a separate index to which the individual neurologic dysfunction scores are helpful adjuncts. The details of the method are outlined in the original report by Kurtzke.[14]

b. *Techniques of quantitative neurologic testing and scoring of data.* Methods of testing individual neurologic functions with apparently greater precision than the standard neurologic examination and employing tools and techniques of motor and sensory testing which give quantitative values of function in terms of numerical units have been investigated by Kuzma and Tourtellotte et al.[15a, b] to determine their feasibility, practicability, and stability under varying conditions in normal subjects. These consist of test procedures for measuring: (1) balance; (2) visual acuity; (3) cutaneous two-point discrimination; (4) vibration sense; (5) coordination of hand; (6) speed of hand and foot movements; (7) muscle strength of selected muscles; and (8) muscle fatigue in selected muscles.

In a pilot study[15a] in which these techniques were used in intact subjects it was found that the methods provided reproducible results in different hands, including those of technicians, students, and different physicians at different times. Preliminary studies have also been carried out on subjects with multiple sclerosis and reported to have reproducible results.[15b]

The potential value of such a system of examination and scoring rests on the following apparent advantages: (1) Greater precision and objectivity in the measurement of neurologic dysfunction as exhibited in the subject's quantitative performance or response. (2) Greater ease of converting test responses into numerical values. (3) Greater reliability of comparisons between functions at different times because of the uniform methods of measurement and objective scoring. (4) Greater efficiency and speed in performing tests of neurologic function. (5) Satisfactory administration of tests by trained paramedical or non-medical personnel. (6) The ready handling of the numerical data obtained from these tests in statistical analysis.

Apparent disadvantages are that all aspects of neurologic function and accessible locations of examination in which dysfunctions might appear are not tested. A limited selection of accessible functions is tested, probably representing areas in which the majority of peripheral neurologic dysfunctions are likely to be located in subjects with multiple sclerosis, but actually governed by the availability of techniques and tools for "quantitative" testing.

As in any method of clinical neurologic testing, the disparity between the activity of the basic disease process and the amount of peripheral neurologic dysfunction is unknown and the correlation between these two variables may be crude. It is questioned whether anything less than gross changes in function are acceptable as reliable evidence of change in the basic disease process. The finer degrees of alteration in peripheral neurologic function to which precise quantitative methods might be sensitive may not be indicators of change in the basic disease process but at times indicate simply physiologic alterations. Further experience with the application of these methods may provide insight into these problems.

For the present it is recommended that for measuring the current status of the disease process and following its course in an experimental trial of therapy, the routine neurologic examination continue to be carried out at intervals and scored as indicated, but that quantitative test procedures be included and evaluated further when feasible.

Assessment of relapses. A characteristic feature of multiple sclerosis is the tendency in many patients for the signs of activity of the disease to wax and wane, sometimes in a pattern of acute attacks of symptoms separated by intervals of improvement. In the literature on multiple sclerosis, marked fluctuations in the condition of patients have been referred to as "exacerbations and remissions," "episodes," "bouts," and "relapses." This feature of the disorder has been of interest not only in connection with the natural history, etiology, and pathogenesis of the disease, but also utilized as a basis for investigating the effects of treatment. In fact, some have held that the only certain way of establishing the efficacy of treatment is through the prevention of relapses.

The major problems associated with the effective utilization of data on relapses are four:

(a) *The uncertain proportion of patients with multiple sclerosis who exhibit this feature.* Brown and Putnam[16] claimed that a third of their cases never showed a remission. Thygesen[17] stated that only half of his patients began with an exacerbating course, and, of these, a third became steadily progressive. Of the half that were chronically progressive from the start, 80 per cent never showed subsequent remissions. In Müller's large series[18] 90 per cent are claimed to have followed a relapsing or intermittent course and the same proportion was found in McAlpine and Compston's series.[13] In the latter series, over half of those with steady progression from the onset had superimposed relapses.

(b) *The wide disagreement among observers regarding the frequency of the phenomenon,* including the alleged tendency for relapse rates to decline progressively as the disease advances. Müller[18] found the relapse rate greatest in the first year, still high during the next five years, and lower thereafter. His finding of an average annual relapse rate of 0.5 over an average period of 9.7 years (in 810 cases with 3797 "bouts") obscures the wide variation in the relapse rate between different individuals; between different phases of the disease in the same individual; and between subjects who have had the disease for varying durations. Thygesen[17] found an attack rate among subjects which was approximately the same regardless of the duration of disease up to 20 years, namely, an average of 1.15 per year. McAlpine and Compston[13] concluded that the average number of relapses per year tended to diminish with time. This was based on a range of average rates of 0.23 to 0.42 per year in cases with up to four years' duration of the disease; 0.31 in cases up to nine years' duration; 0.26 in cases up to 14 years' duration; and 0.2 to 0.22 in cases up to 30 years' duration. Alexander[3b] concluded that relapses occur chiefly during the first five years of the disease. Swank's curve[4] showed an irregular exacerbation rate for cases of varying duration ranging from approximately 1 to 1.6 per year in groups of cases with different average durations up to 10 years.

(c) *The questionable validity of retrospective data obtained from patients concerning previous relapses.* The inherent inaccuracy of such information has posed grave problems in treatment comparisons of the historical variety, i.e., before and after

treatment in the same patient. Experimental trials of therapy utilizing the unreliable data of pretreatment relapse rates based on historical information are therefore not likely to be sound.

(d) *The lack of uniformity or precision in definitions of "relapse."* In the literature this concept has not been clearly enough defined to endow the observations of various investigators with adequate uniformity. If the prevention of relapses is to be utilized in the evaluation of therapy, clarification of terms is essential. Any attempt at defining recurrent bouts shows difficulties and complexities not apparent on the surface.

In a long-range experimental trial of therapy where tabulation of relapse rates might serve a useful purpose for comparison of treatment and control groups, it is obvious that the intensity of disease recrudescence, the duration of periods of disease recrudescence, and the duration of periods of inactivity over a given period of time would also be of significance. If these aspects also are to be measured, they require definition. The terminology and definitions finally adopted should be those which best serve the purpose of recording, tabulating, and analyzing the data for valid evidence of changes in the disease state. To achieve reasonable uniformity of concept, certain arbitrary, though rational, elements have been included in the following definitions:

(1) *Period of worsening (relapse):* This phase represents the period of development of a new symptom or group of symptoms or the period of aggravation of an existing symptom or symptoms if the course has been stationary or improving during the previous one month. To be counted as a separate period of worsening or relapse, the period must last at least 24 hours or longer. In any instance, symptomatic worsening should be counted as a relapse only if it is accompanied by an appropriate change in objective neurologic function as determined by examination.

The duration of a period of worsening is the time elapsing from the beginning of the worsening until symptoms and signs reach their maximum. If symptoms progress after a stationary or improving period of less than one month, this time period is included as part of the duration of the episode of worsening. The latter qualification seems important to avoid the danger of recording minor changes in the course of downhill progression, noted from week to week, as separate relapses or new episodes of disease, thus giving a falsely high numerical score for the relapse rate if this is calculated. In order to utilize relapse rates as a reflection of the course of illness, the attempt must be made to distinguish between minor and questionable degrees of progression and the abrupt flare-ups of disease activity generally implied by the term "relapse." The course reflected by the temporal aspects of disease activity will probably be better shown, however, in the statistical tabulation of the duration of periods of worsening, than merely in their number.

(2) *Period of maximal symptoms (plateau):* This phase extends from the time a symptom or group of symptoms reaches its maximum intensity until improvement begins, or one year has elapsed, whichever occurs sooner, provided that in the

following one month no new symptoms develop. Measuring and recording the duration of the phase of maximal symptoms in each patient may provide additional data reflecting the intensity of the disease, tabulations of which may be of use in following its course.

(3) *Period of improvement (remittent phase):* This period extends from the first evidence of improvement until the end of further improvement. The end of the period of improvement will be considered to be a point, (1) marked by disappearance of symptoms or (2) marked by the beginning of stationary residual symptoms (providing that the periods following (1) or (2) last at least one month); or a point (3) marked by the beginning of an exacerbation of symptoms entering a new period of worsening (relapse). Additional improvement after an inactive period of less than a month will be counted as an extension of the preceding period of improvement. Conversely, if a period of improvement, or of a combined period of improvement and subsequent stationary period is of less than one month and followed by a period of worsening, then the brief period of lessened symptoms will not be so counted but as a continuation of the previous period of worsening.

(4) *Period of inactivity (stationary phase):* This period begins with the end of the period of improvement, but is counted as such only if it lasts more than one month. A shorter stationary period is designated as part of a period of worsening or improvement depending on factors referred to under (3). In the event of persistence of a previous period of worsening at a maximal level without an ensuing period of improvement (i.e., a remittent phase), the so-called period of inactivity is not considered to begin until one year after the worsening reached its maximum. From whatever point the period of inactivity is considered to begin, it extends until new symptoms appear or continuing symptoms worsen.

The above are operational definitions, of necessity somewhat arbitrary, which may not fit every situation encountered in the subjects who are being followed, but will provide a starting basis for classification of changes in the course of the disease. Though it is likely that most of the total time-span involved in the observations can be classified under these several designations, it may be necessary after analyzing the data retrospectively to modify the definitions or to add additional categories in order to clarify the state and magnitude of activity of the disease in time and to provide uniformity of interpretation of the data.

Minor fluctuations of existing symptoms to be excluded from the category of relapses may be defined as improvement or worsening of an established symptom lasting less than 24 hours. Such changes, especially brief ones lasting minutes or hours, may be due to physiologic influences unrelated to the basic disease process, though it is recognized that genuine exacerbations (periods of worsening) of multiple sclerosis probably can last less than 24 hours. Because of the lack of reliable distinguishing criteria, however, and because of the frequency of such transient changes, it is recommended that these phenomena not be tabulated as evidence of renewed or intensified activity of the disease.

Since by the definition given above, a period of worsening is acceptable as a valid datum in an experimental trial of therapy only if confirmed by neurologic examina-

tion revealing objective signs of worsening, the use of historical information concerning relapses is acceptable only tentatively when related in letters or by telephone by subjects who cannot be examined immediately. Before final acceptance of such data, a reported episode of worsening will require corroboration by subsequent examination and finding of objective changes in the neurologic examination. Because of the tendency to forget details with the passage of time, patients serving as subjects in an experimental trial of therapy should be encouraged to report immediately new symptoms lasting longer than a day.

In an experimental trial of therapy it is recommended that records be maintained of the temporal phases of the disease as an index of its activity during the period of observation in treatment and control groups. If a summation in each case of the number of flare-ups, the total number of weeks or months of worsening, and the duration of periods of maximal symptoms were taken into account in the comparison, additional factors reflecting duration of disease activity would be included in the analysis. By what means this data should be integrated might better be determined after preliminary analysis. Lastly, the data derived from the assessment of the neurologic status reflecting extent and severity of pathology may also be integrated with the temporal aspects of worsening in arriving at values representing the true status of the disease at any time during or at the end of a period of observation.

Laboratory Evaluation

Of the many reported clinical pathological alterations in multiple sclerosis such as changes in capillary fragility, platelet adhesiveness, sedimentation rate, serum protein chromatograms, serum fibrinogen level, cerebrospinal fluid phosphorus content, and others, a few have been alleged to fluctuate with the course of the disease. None has been substantiated as varying predictably with disease activity to a degree warranting its routine use in following the course of multiple sclerosis. Though abnormalities in the cellular and protein composition of the cerebrospinal fluid have been thought to reflect activity of the disease, these criteria are not dependable to determine if the disease is in exacerbation or remission. (Quantitative estimations of cerebrospinal fluid gamma-2 globulin content should be repeated at intervals during the course of observation to further explore the significance of this abnormality.)

Changes in visual acuity and abnormalities in the visual fields, cystometrogram, or electroencephalogram should be followed up and repeated at appropriate intervals during the course of the disease, since data from these procedures may provide useful information reflecting aspects of the progress of the disease.

The Experimental Design

The design of the experiment should insure an unbiased and efficient comparison so that nothing in its conduct favors one therapy over another. A successful experimental trial of therapy depends on seeking specific information.[19] Limiting the inquiry to whether one treatment is better than another is too general to guide the development of research plans for an experimental trial. The conditions must first be made specific as to the type of patient, the therapeutic goal and how it is to be measured, the type, dosage, and mode of administration of therapy, the duration of therapy, and the nature of the comparison on which the evaluation of therapeutic benefit will eventually depend. If the treatment involves a new drug its pharmacology must be well worked out so that hazards for particular kinds of patients will be appreciated and consideration may be given to their exclusion from the trial.

Therapeutic Regimens and the Need for Comparison

Comparison of therapies is an essential element of experimental trials, even though one of the regimens may be a mock procedure, placebo, or no special medication. Only rarely can past experience with one regimen provide a valid contrast for a new treatment, thus permitting the new regimen to be administered to all the subjects in an experiment. Simultaneous application of two or more regimens to randomly differentiated subgroups of subjects, with the subgroups exposed to conditions identical in all respects except for the therapeutic agent, is virtually always necessary to provide an unbiased trial of therapy. If there are several treatments to be evaluated it may be more efficient to evaluate them simultaneously than in separate experiments. This is especially true whenever the administration of one would not interfere with the administration of another to the same patient.[20] Since there is no well-established therapy for multiple sclerosis, the experimenter has greater freedom in the use of the placebo, a useful device for eliminating from the estimate of the therapeutic effect any component of the response that rests merely on the meaning of the therapy and of the therapeutic situation for the patient. Even though in the evaluation of changes in multiple sclerosis attention is directed chiefly to objective signs and the placebo effect is chiefly on subjective symptoms, it is pertinent to note that the magnitude of the average placebo effect on subjective symptoms has been estimated as on the order of 35 per cent.[21]

The therapeutic agent should be given in sufficient dosage to fairly represent its full therapeutic potential, with due attention to the possibility of toxicity in the higher range of dose. Although one or more fixed dosage schedules are usually adequate, individual response to certain drugs may be so variable as to require individualization of dose. This need not invalidate the controlled trial, but may limit the interpretation of the results, in that the skill of the therapist then becomes an integral part of the therapeutic situation. Should variation in his skill seem important, efforts to estimate its effect may be considered.

In the course of any experimental trial some multiple sclerosis subjects may encounter difficulties leading to consideration of discontinuing treatment. An "escape clause" in the protocol is essential for the protection of the patient and the physician. To protect the scientific integrity of the therapeutic trial, however, it is essential that such withdrawals be made without regard to the particular regimen the patient may have been assigned, e.g., whether the test regimen or a placebo.

Sample Size

All measurements have a degree of inherent variation dependent on technical or human factors and independent of changes in the value measured. The estimation of this intrinsic variation is accomplished by repeating the observations (replication). Any observed difference between the results in different treatment groups must be referred to this estimate of intrinsic variation in order to determine the probability with which the observed difference might occur by chance between regimens of exactly the same effectiveness. Since the size of the sample is a measure of the amount of replication, the precision of any comparison will increase with sample size and the risks of erroneous inference will decrease.

If the patients are so heterogeneous, however, that the treatment could be effective for some types and not for others, then the subjects must be subdivided accordingly. The estimate of the number of subjects required to detect true treatment differences, if they do exist, then pertains not to the aggregate number treated but to the most meaningful subgroups. If the sampling variability of the material is known, then a required sample size may be calculated on the basis of: (1) the size of smallest change in the subject's condition thought to be important; (2) the

acceptable degree of risk of attributing an advantage to the new treatment it does not possess, i.e., the level of statistical significance to be used in testing the treatment difference observed at the end of the trial; and (3) the degree of acceptable risk that a true treatment effect will go undetected. Since the problem is a common one, there are tables,[22-24] that will shorten the labor of computing sample sizes according to such specifications. Although such calculations often seem quite arbitrary, the "fixed-sample" approach to sample size is the classical one. Investigators frequently prefer to determine sample size in relation to the number of patients available to them, to their costs, etc., but such practical considerations alone will not protect a clinical trial from failure to detect even a large therapeutic effect.

Design of the Treatment Contrast (Planned Comparison of Treatment Results)

To effect a comparison of competing regimens, patients must be grouped in relation to these regimens and possibly to other major factors as well. Such groupings will determine the way in which the analysis of treatment and other effects will be made. A secondary purpose of grouping is to isolate, and usually to measure, the influence of major variables other than treatment so that they will not exaggerate the estimate of experimental error to which the treatment difference will be referred in testing its significance.[25]

There are two main patterns for arranging treatment contrasts.[25, 26, 27] In the first the individual patient receives each treatment under study, and thus serves as his own control. In this way, the heterogeneity among patients is eliminated and the measure of intrinsic variation is derived from changes within patients over time. It seems likely that the spontaneous variability within patients over time gives this pattern little value in designing trials of therapy for multiple sclerosis patients.[28] In the second pattern the treatment comparison is made by comparing physically distinct sets of patients on different treatments. In multiple sclerosis a treatment contrast will probably need to be of this type. If the patients under study were known to be heterogeneous as to treatment response, then it would be advantageous to group patients into more homogeneous blocks so that treatments might then be assigned at random within these blocks.

Another approach to the control of heterogeneity in clinical material is to match cases with respect to variables known to be influential, and then to assign treatments randomly to the members of the matched set. Rarely is enough known about the significant covariables to invest this approach with much efficiency,[29] and in any event the critical question is not whether there is an association between the covariable and the criterion or response variable, but rather how large any correlation is. The correlation must be high (over 0.5) before matching on a covariable is likely to add much precision to the treatment comparison. If patients are becoming available one at a time, such matching will be difficult. If many variables are to be used in matching, a very large series may be required to match a few cases. Therefore, in experimental trials of therapy in multiple sclerosis, matching of cases is impractical.

Allocation of Subjects to Groups

Randomness in the allocation of patients to treatment and control groups guarantees the validity of the comparison and insures that it is unbiased. Various systematic arrangements are often proposed as if they had the property of randomness, but close inspection will always reveal uncertainties or even frank bias. The standard vehicle for achieving randomness is the table of random numbers.[30, 31] All factors not systematically taken into account in the structure of the treatment

comparison, as by controlling the grouping of patients, will be so handled by randomization as not to favor one treatment over another, and to justify the use of random-variable theory in the statistical interpretation of the resulting data, including the measurement of the uncertainties to be attached to the resulting inferences. The allocation by means of random numbers will not, of course, protect against influences arising for the first time after the point of allocation. Only factors present at the time of allocation are effectively randomized.

Observation of "Therapeutic Response"

Specification of the changes in the subject's condition[19, 22] to be measured (i.e., changes in neurologic status, relapse rate, or other features utilized to reflect the course of the disease) is made in the planning stage and the necessary observations defined. Unless there is agreement on this point before the observations are made, there is no guarantee that conclusions will be unbiased and unaffected by the hopes or prejudices of the experimenter. Agreement should extend to specification of the way in which the response variables are to be observed, and how they are to be used in the analysis. If some statistical method of combining the data is to be employed, it should be worked out in advance. A time-limit should be specified for the period of observation, a period considered adequate for the full demonstration of any therapeutic effects. The objective observations which may be utilized for measuring and recording the course of activity of multiple sclerosis have been described above under OBSERVATION OF COURSE OF DISEASE.

The precision of the observations, to which the measurement of intrinsic error is intimately related, affects the power of the experiment to detect treatment differences, as does the closeness of correlation between the features observed and the underlying disease. It is obvious that great power from this source is not available in experimental trials of therapy in multiple sclerosis. It may be more important to increase the sample-size of the experiment than to sharpen the observational techniques. Nevertheless, standardization of the observational techniques, including specification of equipment, examination procedures, and laboratory protocols, will aid precision. These must be worked out and reduced to writing in order that the observations be not only relevant and valid, but also reliable. Recommendations as to these have been made above. It will also be of practical benefit to develop special record forms, scales, check lists, etc. for the clinical trial and to pretest them in an actual clinical situation before placing them into effect.

The setting of the experimental trial needs careful attention so as not to defeat the intent of the randomization. The biasing influence of later events must be avoided, such as knowledge by various individuals participating in the experimental trial of therapy as to what treatment individual subjects are receiving. Unconscious bias may derive from knowledge by the patient, by the physician (experimenter), or by other personnel involved in the observation or recording of data. The value of the so-called "blind" procedures has been amply demonstrated in clinical trials of therapy. Acceptance of the principle, however, is often easier than the achievement of a truly "blind" experiment.[9] Whatever bias may result from failure to attain truly blind conditions will be combined with the apparent treatment effect in a way that will defy separation.

It is recommended that experimental trials of therapy in multiple sclerosis utilize the "double-blind" method. In view of the need to adjust the individual dose, the experimental therapeutic situation may require the operation of two physicians, one with responsibility for dose and the welfare of the patient, the other to serve as unbiased observer. The possibility of feed-back to the observer from the treating physician via the patient must be guarded against should this compromise be necessary.

"Drop-outs" need to be handled with special care if they are not to be a source of bias: in a given situation, interpreted as a threat to the patient's welfare, removal from therapy should in no way be influenced by the particular therapy which the patient is receiving. In general, whatever limitations seem indicated morally, or necessary medico-legally, should be imposed to minimize risk to the patient but in such fashion as to avoid bias from leaks of information to both subjects and experimenter.

Incomplete follow-up is another potential source of bias. In long-term studies patients drift away from observation, and there is ample opportunity for this drifting to be influenced by their reactions to the therapeutic trial. Special efforts are needed to keep follow-up at a sufficiently high level to minimize such error and to investigate its presence in specific trials.

Analysis and Interpretation

The analysis is largely dictated by the design. It provides a test on the hypothesis to which the trial is directed. An early step in the analysis should be the examination of the results of randomizations on salient characteristics of the treatment and control groups. If these characteristics are not similarly distributed within the treatment groups, and there is always a small probability that this will be so, then it will be advisable to take this fact into account in the analysis in some fashion not visualized in the experimental design.

In the analysis of the results of the experiment, the significance tests on differences among treatment groups should he supplemented by estimates of the therapeutic effect, if any, and these estimates stated in the form of confidence intervals. The drop-outs and losses from follow-up introduce possible bias and add uncertainty to the measurement of the treatment effects. If appreciable, they should be included in the analysis in such fashion as to obtain upper and lower limits of treatment effects.

It is rare that a single therapeutic trial will seem sufficient to establish the value of a new method of therapy and, in view of the uncertainties inherent in biological material, it behooves the investigator to be conservative. In addition to the usual risks of false inference, the investigator rarely can be certain that the groups of patients in his trial are representative of the population of patients of interest. The interpretation of the significance tests implies acceptance of risks, most obviously in the significance level itself, .05 or .01. On the other hand, if the results appear not to differ significantly, the power of comparison should be estimated to determine whether the experiment was really powerful enough to have had a good chance of finding a therapeutic effect if there were one.

SUMMARY

Multiple sclerosis, a demyelinative nervous system disease of world-wide occurrence and unknown cause, has been reported to be benefited by various types of therapy based on uncontrolled observations. Such reports have led to unwarranted and occasionally widespread application of methods whose value remained unsubstantiated. Numerous obstacles standing in the way of judging the effects of treatment in this disease have been cited.

Problems of achieving scientific control in experimental trials of therapy in multiple sclerosis were studied and an attempt made to work out procedures which would insure the soundness of such investigations. It was hoped by this means to encourage the application of uniform standards of scientific control in the evaluation of the effects of therapy in this disease.

Problems to which solutions were sought were the following: (1) Selection of subjects, requiring a definition of multiple sclerosis, clinical and laboratory diagnostic criteria, and criteria for exclusion of patients from an experimental investigation of therapy; (2) methods of evaluating the course of the basic disease process (a) through assessment of the neurologic status with emphasis on scoring of the clinical manifestations of the disease and (b) through following the chronologic or longitudinal patterns of activity; (3) the general principles necessary to insure validity and reliability in therapeutic experiments, including statistical methods applicable to the special problems of treatment trials in multiple sclerosis.

Though a specific protocol was not designed, important elements entering into an experimental design were presented and recommendations made regarding their incorporation in a therapeutic trial in multiple sclerosis. It is hoped that the report will provide guidelines for potential investigators of therapy in multiple sclerosis and serve perhaps also as a basis for larger, cooperative trials of therapy among clinical scientists interested in progress in this field.

References

1. Compston, N. D. 1953. Disseminated sclerosis: assessment of the effect of treatment on the course of the disease. Lancet **2**: 271-275.
2. Schumacher, George A. 1960. (a) Multiple sclerosis. Postgrad. Med. **27**: 569-583. (b) Therapy in multiple sclerosis. Internat. J. Neurol.

3a. Alexander, L. 1951. New concept of critical steps in course of chronic debilitating neurologic disease in evaluation of therapeutic response. AMA Arch. Neurol. Psychiat. **66**: 253-271.

3b. Alexander, L., A. W. Berkeley & A. M. Alexander. 1958. Prognosis and treatment of multiple sclerosis — quantitative nosometric study. JAMA **166**: 1943-1949.

3c. Alexander, L., A. W. Berkeley & A. M. Alexander. 1961. Multiple Sclerosis: Prognosis and Treatment. A Nosometric Approach. Charles C. Thomas. Springfield, Ill.

4. Swank, R. L. 1961. A Biochemical Basis of Multiple Sclerosis. Charles C. Thomas. Springfield, Ill.
5. Miller, H., D. J. Newell & A. Ridley. 1961. Multiple sclerosis: treatment of acute exacerbations with corticotrophin (ACTH). Lancet **2**: 1120-1122.
6. Francis M. Forster, Ed. 1961. Evaluation of Drug Therapy. Proceedings of the Symposium on Evaluation of Drug Therapy in Neurologic and Sensory Diseases. University of Wisconsin (May, 1960). University of Wisconsin Press. Madison, Wis.
7. Fisher, R. A. 1951. The Design of Experiments. Sixth Ed. Oliver and Boyd. Edinburgh, Scotland.

8a. Hill, A. Bradford. 1951. The clinical trial. Brit. Med. Bull. **7**: 278-282.

8b. Hill, A. Bradford. 1952. The clinical trial. New Eng. J. Med. **247**: 113-119.

9. Lasagna, Louis. 1955. The controlled clinical trial: theory and practice. J. Chron. Dis. **1**: 353-367.

10a. Beebe, Gilbert W. 1957. Statistics and clinical investigation. Depart. Med. Surg. Med. Bull., Vet. Admin. Washington, D.C.

10b. Beebe, Gilbert W. 1962. Principles of research design in therapeutic trials. Read at the Seminar on Therapeutic Trials. Lynchburg Training School & Hospital (Colony, Va.).

11a. Yahr, M. D., S. S. Goldensohn & E. H. Kabat. 1954. Further studies on gamma globulin content of cerebrospinal fluid in multiple sclerosis and other neurological diseases. Ann. N.Y. Acad. Sci. **58**: 613-624.

11b. Ivers, R. R., B. F. McKenzie, W. F. McGuckin & N. P. Goldstein. 1961. Spinal-fluid gamma globulin in multiple sclerosis and other neurologic diseases. Electrophoretic patterns in 606 patients. JAMA **176**: 515-519.

11c. Schapira, K. & D. C. Park. 1961. Gamma globulin studies in multiple sclerosis and their application to the problem of diagnosis. J. Neurol. Neurosurg. Psychiat. **24**: 121-124.

11d. Foster, J. B. & D. B. Horn. 1962. Multiple sclerosis and spinal fluid gamma globulin. Brit. M. J. **1**: 1527-1528.

11c. TOURTELLOTTE, WALLACE W. 1963. Multiple sclerosis and cerebrospinal fluid. Med. Clin. N. A. 47(6): 1619-1628.
11f. TOMASI, JR., T. B. & G. A. SCHUMACHER. Unpublished observations.
12. ARKIN, H., I. C. SHERMAN & S. L. WEINBERG. 1950. Tetraethyl-ammonium-chloride in the treatment of multiple sclerosis. AMA Arch. Neurol. Psychiat. 64: 536-545.
13. MCALPINE, D., N. D. COMPSTON & C. E. LUMSDEN. 1955. Multiple Sclerosis. E. & S. Livingston, Ltd. Edinburgh, Scotland.
14. KURTZKE, J. F. 1961. On the evaluation of disability in multiple sclerosis. Neurology 11: 686-694.
15a. KUZMA, J. W. 1963. A statistical study of various aspects of a battery of clinical neurologic tests. Ph.D. Thesis. Univ. of Mich. Ann Arbor, Mich.
15b. TOURTELLOTTE, W. W., A. F. HABRER, J. F. SIMPSON & J. W. KUZMA. 1965. Quantitative neurological examination and drug evaluation in multiple sclerosis. This Annal.
16. BROWN, M. R. & T. J. PUTNAM. 1939. Remissions in multiple sclerosis. Arch. Neurol. Psychiat. 41: 913.
17. THYGESEN, P. 1953. The Course of Disseminated Sclerosis. Rosenkilde and Bagger. Copenhagen, Denmark.
18. MÜLLER, R. 1949. Studies on disseminated sclerosis with special reference to symptomatology, course and prognosis. Acta Med. Scandinav. (Supp. 222). 133: 1-214.
19. EDWARDS, ALLEN L. & LEE J. CRONBACK. 1952. Experimental design for research in psychotherapy. J. Clin. Psychol. 8: 51-59.
20. DOLL, R. 1960. The concurrent assessment of several treatments. In council for international organizations of medical sciences (UNESCO and WHO). Controlled clinical trials. Charles C Thomas, Springfield, Ill.
21. BEECHER, HENRY K. 1959. Placebos and the evaluation of the subjective response. *In* The Clinical Evaluation of New Drugs. S. O. Waife & Alvin P. Shapiro, Eds. Harper & Bros. New York, N.Y.
22. MAINLAND, D., L. HERRARA & M. I. SUTCLIFFE. 1956. Tables for use with binomial samples. New York Univ. Coll. Med. New York, N.Y.
23. COCHRAN, WILLIAM G. & GERTRUDE M. COX. 1950. Experimental designs. John S. Wiley & Sons. New York, N.Y.
24. KRAMER, M. & S. W. GREENHOUSE. 1959. Determination of sample size and selection of cases. *In* National Academy of Sciences-National Research Council: Psychopharmacology, Problems in Evaluation. NAS-NRC Publication 583. Washington, D.C.
25. COX, G. M. 1950. The function of designs in experimentation. E. J. deBeer, Ed. The place of statistical methods in biological and chemical experimentation. Ann. N. Y. Acad. Sci. 52: 789-942.
26. FINNEY, D. J. 1955. Experimental design and its statistical basis. Univ. Chicago Press. Chicago, Ill.
27. KEMPTHORNE, O. 1952. The design and analysis of experiments. John S. Wiley & Sons. New York, N.Y.
28. DOLL, R. 1959. Practical problems of drug trials in clinical practice. *In* Quantitative Methods in Human Pharmacology and Therapeutics. D. R. Laurence, Ed. Pergamon Press. London, England.
29. COCHRAN, WILLIAM G. 1953. Matching in analytic studies. Am. J. Public Health. 43: 684-691.
30. FISHER, R. A. & F. YATES. 1949. Statistical Tables for Biological, Agricultural and Medical Research. Hafner. New York, N.Y.
31. RAND CORPORATION. 1955. A million random digits with 100,000 normal deviates. Free Press. Glencoe, Ill.
32. ISBELL, H. 1959. Preliminary clinical assessment of drugs used in mental illness. *In* National Academy of Sciences-National Research Council. Phychopharmacology, Problems in Evaluation. NAS-NRC Publication, 583. Washington, D.C.

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Safety and Tolerabilityof Subcutaneous Cladribine Therapy in Progressive Multiple Sclerosis

ΦII

MS-Scripps

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ABSTRACT: ***Objective:*** To evaluate the safety and tolerability of subcutaneous (s.c.) cladribine therapy in patients with chronic progressive multiple sclerosis (CPMS), and to evaluate the effects on lymphocyte subsets. ***Background:*** Cladribine, a synthetic antineoplastic agent with immunosuppressive effects, may favourably affect the course of CPMS. However results of a previous reported clinical trial showed significant myelosuppression in some patients. ***Design/Methods:*** 19 patients with severe (mean extended disability status score [EDSS] = 6.7) CPMS were treated on a compassionate basis with cladribine 0.07 mg/kg/ day s.c. for 5 days per cycle, repeated every 4 weeks for a total of 6 cycles. Patients underwent clinical evaluation, EDSS, and hematologic analysis before, during, and following therapy. ***Results:*** The treatment was very well tolerated with no clinically significant side effects observed. Between baseline and the end of cycle 6, mean decreases were noted in absolute lymphocyte count from 1697 to 463 ($p = 0.000012$), CD4 count from 865 to 187 ($p = 0.0000008$), CD8 from 418 to 165 ($p = 0.005$) and CD19 from 197 to 26 ($p = 0.000002$). Platelet, granulocyte and RBC counts were unaffected. Approximately one year after completion of therapy, some recovery of CD4 and CD8 counts had occurred although both counts remained suppressed compared to baseline (302 and 227 respectively); the CD19 count had recovered essentially to normal by one year. EDSS scores post-therapy revealed some deterioration in 8 patients and stable scores in the remaining 11. Global patient evaluations of the treatment were mixed. ***Conclusions:*** Cladribine therapy, at lower doses than previously reported, was remarkably well tolerated in CPMS, with no significant myelosuppression. Profound effects occurred in total lymphocyte count and CD4, CD8 and CD19 subsets.

RÉSUMÉ: Sécurité et tolérabilité de la cladribine sous-cutanée dans le traitement de la sclérose en plaques progressive. ***But:*** D'évaluer la sécurité et la tolérabilité de la cladribine sous-cutanée (s.c.) chez les patients atteints de sclérose en plaques progressive chronique (SEPPC) et d'évaluer ses effets sur différentes populations lymphocytaires. ***Introduction:*** La cladribine, un agent antinéoplasique synthétique qui a des propriétés immunosuppressives peut influencer favorablement l'évolution de la SEPPC. Cependant, les résultats des essais thérapeutiques rapportés à date ont montré une myélosuppression significative chez certains patients. ***Méthodes:*** 19 patients atteints de SEPPC sévère (score moyen à l'échelle d'invalidité EDSS = 6.7) ont été traités sur une base humanitaire avec la cladribine à la dose de 0.07 mg/kg/jour par voie s.c. pendant 5 jours par cycle, à toutes les 4 semaines, pour un total de 6 cycles. Les patients ont subi une évaluation clinique, EDSS, et des analyses hématologiques avant, pendant et après le traitement. ***Résultats:*** Le traitement a été très bien toléré, sans effet secondaire cliniquement significatif. Entre la phase pré-traitement et la fin du sixième cycle, des diminutions moyennes du décompte absolu des lymphocytes de 1697 à 463 ($p = 0.000012$), du décompte CD4 de 865 à 187 ($p = 0.0000008$), du décompte CD8 de 418 à 165 ($p = 0.005$) et CD19 de 197 à 26 ($p = 0.000002$) ont été observées. Le décompte des plaquettes, des granulocytes et des globules rouges n'était pas atteint. Environ un an après la fin du traitement, une récupération du décompte CD4 et CD8 était évidente, bien que ces deux décomptes demeuraient supprimés en comparaison avec ceux de la phase pré-traitement (302 et 227 respectivement); le décompte CD19 était revenu à la normale à un an. Les scores EDSS post-traitement ont montré une détérioration chez 8 patients et des scores stables chez les 11 autres. L'évaluation globale du traitement par les patients était mixte. ***Conclusions:*** La cladribine s.c., à dose plus faible que dans les études rapportées antérieurement, a été remarquablement bien tolérée chez les patients atteints de SEPPC, sans myélosuppression significative. Des effets marqués ont été notés sur le décompte lymphocytaire total et sur les sous-populations CD4, CD8 et CD19.

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BACKGROUND

There is considerable evidence that cell mediated immunity plays an important role in the pathogenesis of multiple sclerosis (MS). Helper (CD4) T lymphocytes are found in MS lesions

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along with abnormal MHC class II expression.[1,2] In experimental allergic encephalomyelitis (EAE), injection of myelin basic protein (MBP) and other myelin proteins results in T-cell infiltration into the CNS, accompanied by CNS lesions similar to those seen in MS.[3] T lymphocytes specific to such myelin antigens have been shown to induce CNS inflammation in several mammalian species.[4] T lymphocyte clones reactive to MBP have also been found in the blood of patients with MS.[5] Despite these observations the exact mechanisms of demyelination are unclear.

Beta interferons have been shown to reduce the frequency and severity of exacerbations in the relapsing remitting form of MS.[6] However, little progress has been made in altering the natural history of the disease particularly in patients with chronic progressive MS. Despite early encouraging results, immunosuppressive agents such as cyclophosphamide, azathioprine, and cyclosporin have demonstrated, at best, only marginal activity in double blind controlled trials.[7,8,9]

Cladribine (2 - chlorodeoxyadenosine) is a purine analog which is incorporated into DNA and is resistant to the enzyme adenosine deaminase.[10] It has demonstrated considerable antineoplastic activity in hairy cell leukemia, chronic lymphocytic leukemia and certain forms of non-Hodgkin's lymphoma.[11-14] It has significant immunosuppressive effects, with reduction in the numbers of CD4 and CD8 lymphocytes[10-13] which persist for 6-12 months or more after a course of therapy. The drug is generally well tolerated with the major toxicity being myelosuppression.[10,15]

Recently a small (n = 51), randomized, double-blind, placebo controlled, cross-over trial was reported using intravenous cladribine in patients with CPMS.[16] 48 patients entered as matched pairs and the trial was stopped after one year of treatment before the cross-over occurred. Treatment consisted of four monthly cycles of 0.7 mg/kg cladribine given through a central line. Cladribine appeared to favourably influence the course of CPMS, with improvement or stabilization in neurological scores, lesion volumes on MRI, and concentrations of oligoclonal bands in cerebrospinal fluid in treated patients, compared to placebo. However, although the treatment was generally well tolerated, significant hematologic toxicity was reported, in addition to several viral infections.[15]

Subcutaneous cladribine has shown good bioavailability compared to the intravenous route, with a similar pharmacokinetic profile.[14] Our objective was to evaluate the safety and tolerability of subcutaneous cladribine therapy in patients with chronic progressive multiple sclerosis, and to assess if lower doses than those previously used would be immunosuppressive with less myelosuppression.

Patients and Methods

19 patients (13 females and 6 males) with chronic progressive MS (CPMS) attending the MS Clinic at St. Michael's Hospital in Toronto were treated. EDSS scores ranged from 5.5 to 8, and ages from 31 to 60 years (mean age 43). Patients were selected for treatment on compassionate grounds based primarily on rapid progression in the two years prior to therapy.

The average disease duration in these patients was 12.6 years. 15 patients had no comorbid medical conditions. The following conditions were found in one patient each: asthma, insulin-dependent diabetes mellitus (IDDM), depression, and IDDM with depression. Most patients had at some point in their disease been treated with short term high dose corticosteroids for MS exacerbations. Apart from brief courses of corticosteroids, no patient had received immunosuppressive therapy in the year prior to the study. No patient received concomitant corticosteroid or other immunosuppressive therapy while on cladribine. Cladribine (Leustatin7®, Ortho-Biotech) was administered at a dose of 0.07 mg/kg/day by subcutaneous injection for 5 days per cycle, or 0.35 mg/kg/cycle, repeated every 4 weeks for 6 cycles in total. Complete blood count (CBC) and differential; as well as clinical assessment, were done prior to each treatment cycle; CBC was repeated at day 14 following at least the first cycle to assess the nadir counts. Total lymphocyte counts and CD4, CD8 and CD19 positive lymphocyte subsets were determined prior to initiation of treatment, then at Cycle 3 and 6, and (in most instances) at one year following completion of therapy. Lymphocyte subset analysis was done by immunophenotyping using a FACScan flow cytometer. The normal reference ranges for total lymphocyte count, CD4, CD8 and CD19 subsets were 1500 – 2900, 535 – 1125, 300 – 810 and 135 – 447 x 10^6/L respectively.

Neurologic assessments and EDSS scores were performed by neurologists at the MS clinic at baseline, during therapy, after completion of the 6 cycles, and in follow-up over the next 21 months. Because of difficulties involved in getting significantly disabled patients to return for follow-up, the exact timing of the EDSS assessment varied somewhat.

Data are presented as mean "± standard deviation. The Student's t-test for paired data was used to compare observations; a significance level of 0.05 was used to indicate statistical significance.

Results

Of the 19 treated patients, 13 received all six cycles of cladribine. Six patients chose not to complete therapy, 2 patients after 5 cycles, 3 after 4 cycles and 1 after 3 cycles. The primary reasons patients gave for not completing therapy were perceived lack of efficacy together with the medication cost. Toxicity did not limit treatment in any of the cases.

Laboratory data from 4 patients (patients 2, 8, 10 and 13 on Table 3) were excluded from analysis because of absent baseline lymphocyte subset data in two cases, and insufficient follow-up data in the other two. The total lymphocyte count and CD4, CD8 and CD19 lymphocyte subsets at baseline (prior to the start

Table 1: Lymphocyte subset analysis during therapy (n = 15).

	Baseline	3 Months	p value*	6 months	p value**
Lymphocyte count	1697 ± 570†	801 ± 350	0.0000007	463 ± 207	0.000012
CD4 count	865 ± 313	411 ± 170	0.000005	187 ± 94	0.0000008
CD8 count	418 ± 170	248 ± 145	0.00002	165 ± 127	0.005
CD19 count	197 ± 104	25 ± 27	0.000002	26 ± 16	0.4

P value derived from Student's t test for paired data

*Baseline vs. 3 months

**3 months vs. 6 months

†All values expressed as mean ± standard deviation, x 10^6/L.

of cladribine therapy), and at 3 and 6 months on therapy in the 15 evaluable patients are summarized in Table 1. As shown, significant decreases in total lymphocyte counts as well as in helper (CD4+) and cytotoxic/suppressor (CD8+) lymphocyte subsets were seen during cladribine therapy. There was a continuing decline in T lymphocyte subsets from 3 to 6 months; this was particularly true for the CD4 subset. Highly significant decreases in the B lymphocyte (CD19+) subset was also seen with trough values attained at 3 months.

Follow up laboratory data, one year after completion of cladribine, were available on 12 of these 15 patients and are summarized in Table 2. The mean total lymphocyte, CD4 and CD 8 counts had shown some recovery compared to the values at the end of therapy, but were still significantly below baseline level. The mean CD19 count had recovered to normal levels.

Table 2: Lymphocyte subset analysis following completion of therapy (n=12).

	6 Months	1 year post therapy	p value*
Total Lymphocyte count	475 ± 200†	895 ± 367	0.0003
CD4 count	199 ± 97	302 ± 133	0.018
CD8 count	156 ± 91	227 ± 142	0.047
CD19 count	28 ± 16	179 ± 110	0.00014

*P value derived from Student's t test for paired data
†All values expressed as mean ± standard deviation, x 10^6/L.

Table 3: Summary of EDSS scores obtained on cladribine therapy; 0 months represents baseline (n = 19).

Pt. #	cycles	Months from start of therapy									
		0	3	6	9	12	15	18	21	24	27
1.	6	7.5							8.5		
2.	6	7								7	
3.	6	6			6	6					
4.	6	8	8				8.5		8.5		
5.	6	8					8.5				
6.	6	6.5	6.5	6.5	6.5	6.5					
7.	6	6.5	6.5	7			7	6.5			
8.	6	6.5							6.5	7	
9.	6	6			6	6					6.5
10.	6	6.5			6.5	6.5					
11.	6	6		6			6.5	6			
12.	6	6.5		6.5				7			
13.	4	7		7			7		7.5		
14.	6	7		7			7		7		
15.	5	7			7	7	7				
16.	5	7					7				
17.	4	7.5	8.5					8.5			
18.	4	6.5	6.5					7			
19.	3	5.5	5.5	5.5							

There were no opportunistic infections seen either during cladribine therapy or in the following year. The hemoglobin, granulocyte and platelet counts were within normal reference ranges throughout the duration of cladribine therapy. One female patient developed a borderline anemia with a hemoglobin of 117 g/L while on cladribine which recovered to normal post therapy. Another patient had a mild thrombocytopenia with a platelet count of 136 x10^9/L, after one cycle of cladribine which returned to normal by the subsequent 2 cycles.

No definite nonhematologic toxicity was reported or observed. No patient required a reduction of cladribine dose or treatment delay secondary to adverse effects.

Details of EDSS scores are in Table 3. A significant change in EDSS score was defined as a change of one-half point or more measured at the first post therapy visit and compared to baseline. Follow-up data are available in all patients, at times varying from 6-21 months after completion of therapy. Of these, 8 patients had an EDSS score that increased significantly as compared to baseline, while 11 were unchanged. None had a significantly lower EDSS. Patient global rating scores obtained approximately 1 year after therapy indicated that 5 patients felt they were doing better, 3 were unchanged and 9 worse; the remaining 2 were uncertain.

Discussion

Cladribine is recognized to have significant immunosuppressive effects, characterized by marked reductions in T and B lymphocyte subsets, when used in the treatment of hematologic malignancies. Myelosuppression is the major toxicity. In the original report of cladribine treatment in MS, a statistically significant drop in blood counts was observed.[15] In 7 patients, the platelet count dropped below 80 x10^9/L, while a substantial and sustained decrease in granulocytes was seen.[15] Two patients developed severe and prolonged aplastic anemia requiring red cell and platelet transfusions. In one case, the patient had received prior therapy with carbamazepine and was receiving phenytoin while on cladribine. The second patient had previously received extensive therapy with chlorambucil. Both recovered after several months of marrow suppression. Two patients developed herpes zoster which subsided rapidly on acyclovir treatment. One patient presented with acute fulminant hepatitis B infection 3 days after her second cladribine infusion and died 5 days after admission. She had negative hepatitis B serology at start of therapy and a history of probable recent exposure.

Our series of patients received a lower total treatment dose (total of 2.1 vs 2.8 mg/kg, as well as a lower treatment dose per cycle (0.35 mg/kg vs. 0.7 mg/kg). Using this dosing regimen, patients experienced no significant myelosuppression or infectious problems despite achieving profound lymphocyte suppression. When compared to the higher dose regimen, the rate of decline in the CD4 count using our regimen was less rapid, although the trough CD4 count at six months into treatment was similar.[15] In contrast, the rate of decline, nadir and post-therapy levels of CD8 and CD19 counts were similar in the two groups. At approximately 1 year post-therapy, we noted a partial but incomplete recovery in CD4 counts, while CD4 levels remained severely depressed in the higher-dose study.[15] In view of the presumed pathogenetic role of T helper cells in MS,[1,2] the slower decline and earlier recovery in these cells could have implications

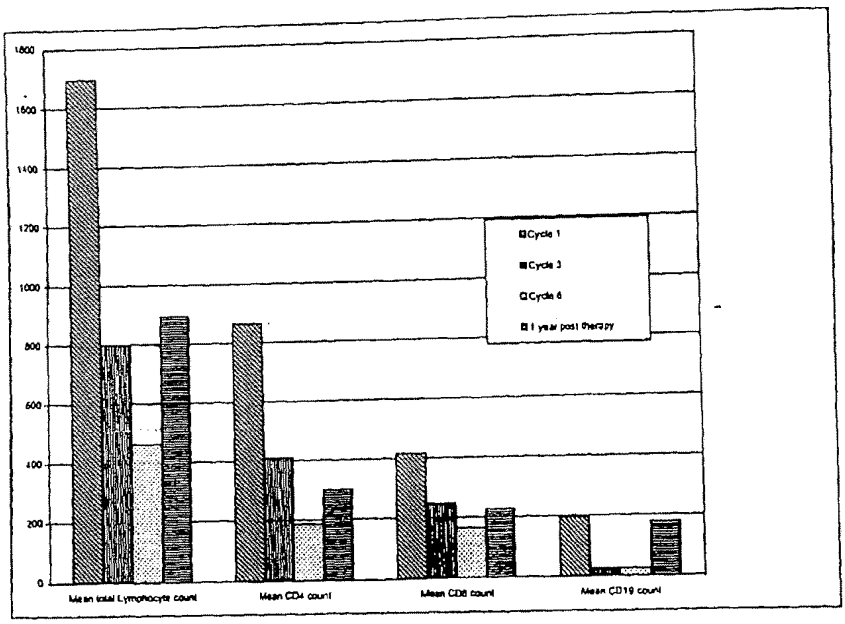

Figure 1: Absolute lymphocyte count and lymphocyte subsets - CD4, CD8 and CD19 at baseline, 3 months and 6 months while on Cladribine therapy, and 1 year after completion of Cladribine.

regarding therapeutic efficacy. However, since only a small subset of T cells is likely involved in producing MS, these implications are unclear. Measuring T lymphocytes reactive to myelin basic protein[5] could address this question *in vitro*, although only a randomized trial could accurately assess the clinical relevance of the effects of the different dosing regimens.

In addition to the lower cladribine dose, none of our patients were on concomitant immunosuppressive or myelosuppressive therapy which may have contributed to the lack of toxicity. Concomitant use of corticosteroids and purine analogs has been associated with opportunistic infections.[17] Whether cladribine is safe to use along with or soon after medications such as beta-interferon, methotrexate, azathioprine or cyclophosphamide is unclear and requires further study. The long-term safety of cladribine in MS is also unknown.

The subcutaneous route of administration has been shown to have a favorable pharmacokinetic profile, with 100% bioavailability and no local toxicity.[14] Such treatment is easy to administer, not requiring intravenous access. Although given in our Medical Day Care outpatient unit, there is no reason in principle why patients could not be trained in self-administration of the medication.

Subcutaneous cladribine therapy, at the doses used in this study, is remarkably well tolerated in chronic progressive multiple sclerosis, with no significant toxicity despite achieving profound and long lasting immunosuppression. The degree of suppression of lymphocytes was similar to the higher-dose regimens, although differences were noted in the rate of decline and recovery of CD4 counts.

As this was a safety and tolerability study with no control group, nothing meaningful can be stated regarding the observed EDSS changes, given the unpredictable course of MS. Although no objective improvements were noted in any patient, we cannot exclude the possibility that cladribine may have contributed to disease stabilization in some instances. We await the results of a large appropriately powered randomized blinded trial of this medication with interest. Although safe and easy to use, the therapeutic effectiveness of cladribine in chronic progressive MS remains to be established.

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REFERENCES

1. FrenchConstant C. Pathogenesis of multiple sclerosis. Lancet 1994; 343(8892): 271-275.
2. Traugott U. Multiple sclerosis: relevance of class I and class II MHCexpressing cells to lesion development. J Neuroimmunol 1987; 16(2): 283-302.
3. Zamvil SS, Steinman L. The T lymphocyte in experimental allergic encephalomyelitis. Ann Rev Immunol 1990; 8: 579-621.
4. Linington C, Berger T, Perry L, et al. T cells specific for the myelin oligodendrocyte glycoprotein mediate an unusual autoimmune inflammatory response in the central nervous system. Eur J Immunol 1993; 23(6): 1364-1372.
5. Allegretta M, Nicklas JA, Sriram S, Albertini RJ. T cells responsive to myelin basic protein in patients with multiple sclerosis Science 1990; 247(4943): 718-721.
6. Anonymous. Interferon beta1b is effective in relapsingremitting multiple sclerosis. Clinical results of a multicenter, randomized, doubleblind, placebocontrolled trial. The IFNB Multiple Sclerosis Study Group. Neurology 1993; 43(4): 655-661.
7. Ebers GC. Treatment of multiple sclerosis. Lancet 1994; 343(8892): 275-279.
8. Anonymous. The Canadian cooperative trial of cyclophosphamide and plasma exchange in progressive multiple sclerosis. The Canadian Cooperative Multiple Sclerosis Study Group. Lancet 1991; 337(8739): 441-446.

9. Anonymous. Efficacy and toxicity of cyclosporine in chronic progressive multiple sclerosis: a randomized, double blinded, placebo controlled clinical trial. The Multiple Sclerosis Study Group. Ann Neurol 1990; 27(6): 591-605.
10. Guchelaar HJ, Richel DJ, Schaafsma MR. Clinical and toxicological aspects of the antineoplastic drug cladribine: a review. Ann Hematol 1994; 69: 223-230.
11. Tallman MS, Hakimian D, Variakojis D, et al. A single cycle of 2chlorodeoxyadenosine results in complete remission in the majority of patients with hairy cell leukemia. Blood 1992; 80(9): 220-239.
12. Juliusson G, Liliemark J. High complete remission rate from 2chloro2'deoxyadenosine in previously treated patients with Bcell chronic lymphocytic leukemia: response predicted by rapid decrease of blood lymphocyte count. J Clin Oncol 1993; 11(4): 679-689.
13. Kay AC, Saven A, Carrera CJ, et al. 2Chlorodeoxyadenosine treatment of lowgrade lymphomas. J Clin Oncol 1992;10(3): 371-377.
14. Liliemark J, Albertioni F, Hassan M, Juliusson G. On the bioavailability of oral and subcutaneous 2-chloro-2'-deoxyadenosine in humans: alternative routes of administration J Clin Oncol 1992; 10: 1514-1518.
15. Beutler E, Koziol JA, McMillan R, Sipe JC, Romine JS, Carrera CJ. Marrow suppression produced by repeated doses of cladribine. Acta Haematol 1994; 91: 10-15.
16. Sipe JC, Romine JS, Zozlol JA, et al. Cladribine in treatment of chronic progressive multiple sclerosis. Lancet 1994; 344: 9-13.
17. Cheson BD. Infectious and immunosuppressive complications of purine analog therapy. J Clin Oncol 1995; 13: 2431-2448.

including a large area of abnormal uptake at the base of the skull, consistent with metastatic disease. Plain radiographs demonstrated extensive erosive lesions at the base of the skull. Needle biopsy of the hepatic lesion demonstrated large-cell undifferentiated carcinoma with "squamoid" features. After tracheostomy and gastrostomy, palliative chemotherapy and radiation therapy were given.

Discussion. The genioglossus, an extrinsic tongue muscle, is stronger than the intrinsic tongue muscles.[3] When the tongue is forcibly protruded, action of the weaker intrinsic tongue muscles may be masked. The intrinsic muscles that turn the tip of the tongue are the superior and inferior longitudinal muscles.[3,4] Unilateral contraction of these muscles shortens the tongue ipsilaterally, turning the tip to that side.

Afer unilateral denervation, the protruded tongue deviates to the weak side. However, the tip of the nonprotruded, unilaterally weak tongue can be turned to the normal side, but not to the weak side. This pattern on protrusion, a sign of unilateral extrinsic muscle weakness, has been well described and illustrated. The sign of intrinsic muscle weakness in the unilaterally weak, nonprotruded tongue may be known to some experienced clinicians, but has not been illustrated in the neurologic literature.

Finally, a unilaterally weak tongue protrudes the cheek on the weak side by using the contralateral genioglossus muscle, not by using ipsilateral intrinsic tongue muscles to turn the tongue into the cheek.

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References

1. Wartenberg R. Diagnostic tests in neurology: a selection for office use. Chicago: The Year Book Publishers, 1953.
2. Haymaker W. Bing's local diagnosis in neurological diseases, 15th ed. St. Louis: C.V. Mosby, 1969.
3. DeJong RN. The neurologic examination: incorporating the fundamentals of neuroanatomy and neurophysiology, 4th ed. Hagerstown, MD: Harper and Row, 1979.
4. Warwick R, Williams PL, eds. Gray's anatomy, 35th British ed. Philadelphia: W.B. Saunders, 1973.

A neurologic rating scale (NRS) for use in multiple sclerosis

Article abstract—A neurologic rating scale (NRS) has been developed for clinical assessment of MS patients. The scale has been tested on 250 MS patients. Assignment of the NRS score is based on assessment of each component of the neurologic examination and accurately reflects overall neurologic function. Clinical exacerbations are evident as significant deviations from baseline scores. There was close interexaminer correlation, with the range of variability no greater than 2.6%. The NRS is a simple, reliable, and sensitive scale that can be used with other objective measurements of neurologic function, such as neurophysiologic studies, in the clinical assessment of MS patients.

NEUROLOGY (Cleveland) 1984;34:1368-1372

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Multiple sclerosis presents unique and difficult problems in the long-term assessment of patients because there are neither diagnostic tests nor reliable laboratory indicators of disease activity. Changes in clinical status have been the principal means for evaluating improvement and assessing new forms of therapy. Several rating scales have been developed to assess neurologic disability and function in attempts to quantify changes in clinical status.[1-5] Refinements of these rating scales have also been proposed.[6-8] The scales are based primarily on activities of daily living rather than on the standard neurologic examination, and they are insensitive to many changes in neurologic function; some are too elaborate for efficient use.

Increased interest in clinical trials[10-13] demonstrates the need for a simple, reliable, sensitive, and clinically reproducible scale. We describe a neurologic rating scale (NRS) that was developed in conjunction with a standard neurologic examination protocol.

Materials and methods. *Patients.* We studied 250 MS patients who fulfilled the conventional diag-

nostic criteria.[9,14,15] Twenty-four patients with the exacerbating-remitting form of MS were enrolled in a clinical treatment protocol and were examined at least 15 times each year for 3 years. Other patients were examined at 3-month intervals or more frequently during periods of clinical fluctuation.

Neurologic examination protocol. A neurologic history and examination form (figure 1) was completed by a neurologist experienced in the evaluation of MS patients. Normal neurologic function was graded as zero, with 1+, 2+, 3+, and 4+ indicating increments of activity (mild, moderate, severe, or maximally increased), and grades of −1, −2, −3, and −4 indicating decrements of functional activity (mild, moderate, severely reduced, or absent). The grading system was applied to mental status, cranial nerves, motor system, sensation, and tendon reflexes.

Neurologic rating scale (NRS). The assignment of points in the NRS directly reflects the examiner's clinical assessment of each component in the neurologic examination (table 1). An intact system receives the full "normal" point value, with a progressive loss in points for mild (−1; 1+), moderate (−2; 2+), or severe (−3, −4; 3+, 4+) involvement. Severe (−3; 3+) and maximal (−4; 4+) deficits are scored as severe on the NRS. A category for important subjective symptoms such as bladder, bowel, or sexual dysfunction was incorporated, because there is no simple way to measure central autonomic function. The total point distributions for the several systems are specifically weighted for common fluctuating neurologic abnormalities of MS, such as visual, motor, sensory, and cerebellar signs. Tendon reflexes and Babinski responses, more often present than other signs, are less emphasized. Disorders of cognition, affect, and mood are also included. The final score is obtained by noting the assigned points in each of the columns and adding the subtotals. A neurologically normal individual would have a score of 100 points.

Results. The NRS has been applied to 250 MS patients. Serial NRS scores for six MS patients are provided (figure 2). The numerical value for each patient encounter measures residual neurologic function. Clinically significant exacerbations were manifested as negative deviations from baseline values, and clinical improvement led to an increase on the NRS scale (eg, figure 2, patient A). Kurtzke Disability Status Scores (DSS)[4] provide a correlation with the status of the patient but were less sensitive to clinical changes than were NRS scores (figure 2, patients A-F).

Four neurologists independently scored the NRS

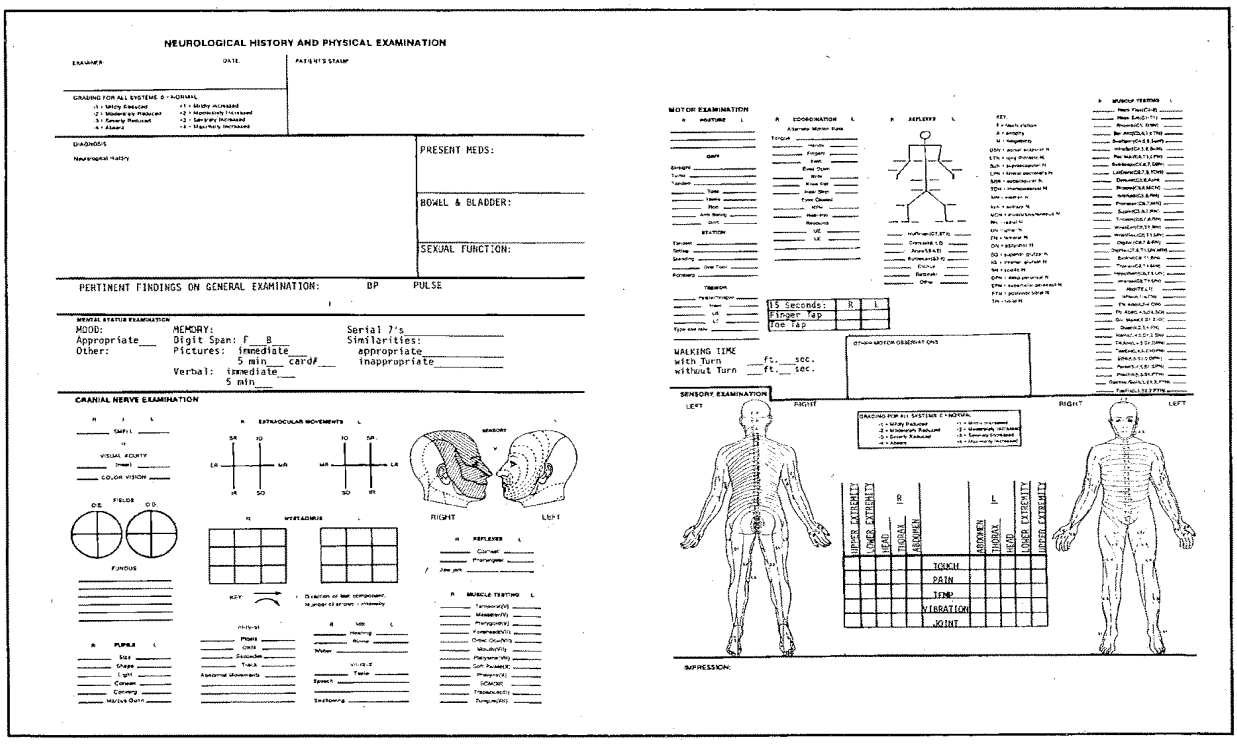

Figure 1. Neurologic history and examination form completed by the examining neurologist at each patient encounter. The grading convention for all systems is noted at the upper left corner of the form.

Table 1. Scripps Neurological Rating Scale (NRS) worksheet*

System Examined		Maximum Points	Normal	Degree of Impairment Mild	Mod.	Severe
Mentation and Mood		10	10	7	4	0
Cranial Nerves:	Visual Acuity	21	5	3	1	0
	Fields, Discs, Pupils		6	4	2	0
	Eye Movements		5	3	1	0
	Nystagmus		5	3	1	0
Lower Cranial Nerves		5	5	3	1	0
Motor:	RU	20	5	3	1	0
	LU		5	3	1	0
	RL		5	3	1	0
	LL		5	3	1	0
DTRS:	UE	8	4	3	1	0
	LE		4	3	1	0
Babinski: R; L (2 ea)		4	4	—	—	0
Sensory:	RU	12	3	2	1	0
	LU		3	2	1	0
	RL		3	2	1	0
	LL		3	2	1	0
Cerebellar:	UE	10	5	3	1	0
	LE		5	3	1	0
Gait; Trunk and Balance		10	10	7	4	0
Special Category: Bladder/Bowel/Sexual Dysfunction		0	0	−3	−7	−10
Totals		100				
Neurological Rating Scale Score						

* Points assigned for each component of the neurologic examination are subtotaled, and points for autonomic dysfunction are subtracted, leaving the final (NRS) score.

for five individual patients, using the neurologic history and examination form that had been completed by an examining physician; the resulting NRS scores were in close agreement (table 2), with a range of variability less than 2.6%.

Discussion. The MS NRS has been introduced and tested as a clinical indicator in the evaluation of patients with MS. It provides a rapid summation of neurologic function as objectively measured by the neurologic examination (figure 1) and, in practical terms, provides a convenient quantitative base of information (table 1 and figure 2) for neurologic functions of MS patients who are followed serially.

NRS scores are more sensitive indicators of clinical change than the Kurtzke DSS, allowing for rapid recording of clinical changes that may not be identified in the DSS (figure 2). Isolated new clinical findings during an exacerbation, such as internuclear ophthalmoplegia, can make up to a 10-point change in the NRS score without altering the DSS. For example, patients D and E (figure 2) had 17-point and 20-point declines in NRS scores, while DSS scores did not change. Similarly, the NRS score of patient A (figure 2) revealed a 17-point improvement, while the DSS was unchanged. Analyses based on the DSS alone may mask important changes in neurologic function.

The NRS is not intended to replace the DSS, but is a more sensitive, complementary clinical method. It is suited for clinical studies with serial observation and may be used with other objective measurements such as neurophysiologic studies, spinal fluid examinations, or other laboratory data. The NRS can be used in either a prospective or retrospective manner, depending on the study design. Because it is simple,

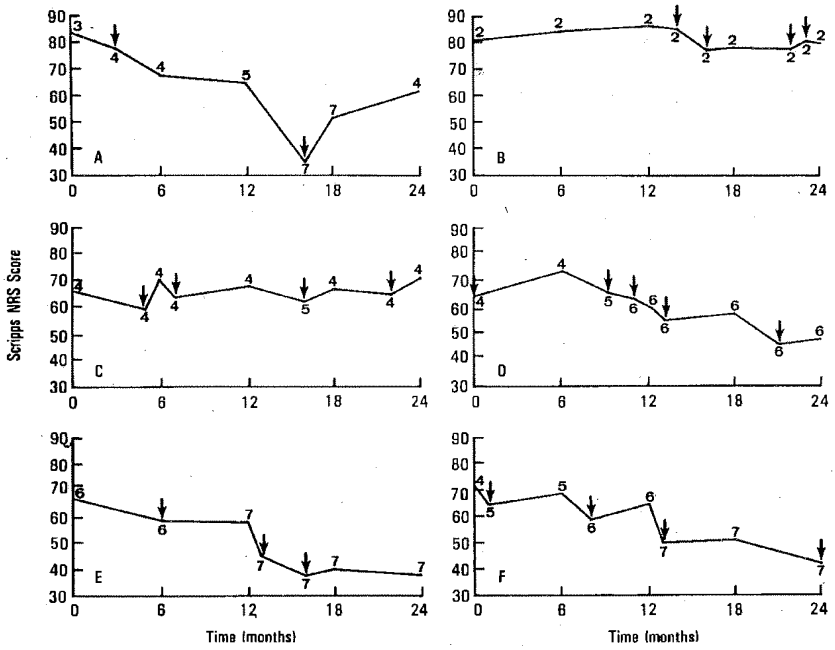

Figure 2. Composite illustration of the clinical course for six patients (A-F). The solid line represents the NRS score, and arrows indicate clinical exacerbations of MS. Kurtzke DSS scores are indicated numerically below *the line during exacerbations and* above *the NRS line during periods of clinical stability or improvement.*

Table 2. Comparison of Scripps NRS scores by four neurologists*

Pt	Dr. 1	Dr. 2	Dr. 3	Dr. 4	Mean ± SD
1	98	96	98	94	96.5 ± 1.9
2	86	81	81	84	83.0 ± 2.5
3	65	67	65	64	65.3 ± 1.3
4	74	70	72	68	71.0 ± 2.6
5	53	56	54	52	53.8 ± 1.7

* Each physician independently scored the same neurologic examination (previously recorded by another physician) for each of five individual patients.

the NRS may also be used to follow the course of disease or the effectiveness of treatment in non-research patients.

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References

1. Kurtzke JF. On the evaluation of disability in multiple sclerosis. Neurology (Minneap) 1961;11:686-94.
2. Kurtzke JF. Further notes on disability evaluation in multiple sclerosis with scale modifications. Neurology (Minneap) 1965;15:654-61.
3. Kurtzke JF. A proposal for a uniform minimal record of disability in multiple sclerosis. Acta Neurol Scand [Suppl] 1981;64:110-29.
4. Kurtzke JF. A new scale for evaluating disability in multiple sclerosis. Neurology (Minneap) 1955;5:580-3.
5. World Health Organization. International classification of impairments, disabilities, and handicaps. Geneva: World Health Organization, 1980.
6. Granger CV. Assessment of functional status: a model for

multiple sclerosis. Acta Neurol Scand [Suppl] 1981;64:4047.
7. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology (Cleveland) 1983;33:1444-52.
8. Mickey MR, Myers LW, Clark O, Ellison GW. An illness severity score for multiple sclerosis. Neurology (Cleveland) 1983;33(Suppl 2):72.
9. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227-31.
10. Hauser SL, Dawson DM, Lehrich JR, et al. Intensive immunosuppression in progressive multiple sclerosis. N Engl J Med 1983;308:173-80.
11. Fischer BH, Marks M, Reich T. Hyperbaric-oxygen treatment of multiple sclerosis. N Engl J Med 1983;308:181-6.
12. Mertin J, Kremer M, Knight SC, et al. Double-blind controlled trial of immunosuppression in the treatment of multiple sclerosis: final report. Lancet 1982;2:351-4.
13. Aronson SM, Bauer HJ, Brown JR, et al. Therapeutic claims in multiple sclerosis. International Federation of Multiple Sclerosis Societies, 1982.
14. Schumacker GA, Beebe GW, Kibler RF, et al. Problems of experimental trials of therapy in multiple sclerosis. Ann NY Acad Sci 1965;22:552-68.
15. McDonald WI, Halliday AM. Diagnosis and classification of multiple sclerosis. Br Med Bull 1977;33:4-9.

Transient cataplexy after removal of a craniopharyngioma

Article abstract—We studied a patient with cataplexy secondary to a surgical lesion that involved the perichiasmal hypothalamus. We believe that this lesion interfered with the hypothalamic mechanism for *timing* sleep and wakefulness, whereas the pontine mechanism for *generating* sleep cycles remained relatively intact.

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Narcoleptic patients (whether falling asleep at night or inappropriately during the day) frequently begin sleep with a rapid eye movement (REM) period. Cataplectic attacks are polygraphically indistinguishable from REM sleep and often develop into full-blown REM sleep episodes.[1,2] The etiology is unknown but may become better understood by study of symptomatic cases that follow anatomically discrete structural lesions of the brain, as in the following case.

Case report. A 14-year-old girl was admitted in March 1966 for evaluation of metabolic bone disease; she had slipped femoral capital epiphyses at age 12 and an atraumatic left tibial fracture at age 14. She had developed normally, except that she was always below average in height. Occasional frontal headaches had appeared at age 13, and she required glasses for reading. Menstruation had not occurred. She weighed 49.8 kg (75th percentile) and was 140 cm in height (5th percentile). Pubic hair was present, and there was early breast development without areolar pigmentation. Examination was normal except for diminished visual acuity in the right eye (20/80) and bitemporal visual field defects. Skull films revealed an enlarged sella turcica (3 cm in maximal dimension), demineralized posterior clinoid processes, and extensive suprasellar calcification. EEG showed paroxysmal bursts of bilaterally synchronous high-voltage theta activity without lateralizing or focal features.

At surgery, a huge tumor was found to occupy the suprasellar region. The entire optic chiasm was forced upward to the left, the right optic tract appeared destroyed, and the mass extended upward in the midline behind the chiasm, pushing the third ventricle upward and backward. The tumor was removed completely; pathologic examination revealed craniopharyngioma and hypothalamic neurons. Postoperatively, her visual acuity was 20/500 in the right eye and 20/200 in the left eye, and she had a left homonymous hemianopia.

She was discharged taking phenytoin. In the next year, she required thyroxine, Pitressin Tannate, cyclic estrogens, and hydrocortisone during periods of stress or infection. In 1967, she returned to school. Her full-scale IQ was 108.

Description of sleep disturbance. In October 1968, she began to fall asleep in class, sometimes suddenly and without warning. In December 1970, she had "fainting" spells that lasted 60 to 90 seconds with apparent unconsciousness and loss of postural tone. These increased in frequency from once weekly to several daily by early 1971. In some attacks, she had hallucinoid dreams. There was no family history of sleep disturbance. Examination was unchanged, except for appearance of bilateral optic atrophy; she weighed between 55 and 65 kg and was 148 cm in height. EEG revealed frequent paroxysmal bursts of diffuse bilaterally synchronous high-voltage theta activity; spike discharges were occasionally seen not confined to the operative area. Her symptoms were unchanged after trials of phenytoin, phenobarbital, and ethosuximide in varying dosages and combinations.

She was readmitted in February and April of 1971. In the attacks, she lost postural tone without warning. If she were standing, she would gradually fall to the floor; if she were in bed, her head would fall back onto the pillow. Eyes were closed, but rapid movements of the globes could be seen beneath the lids. Tendon reflexes were not obtained. No movements, incontinence, injury, cyanosis, diaphoresis, or altered pulse or respiration were seen. She would awaken immediately if spoken to or pinched, apparently aware that she had had a spell. She often reported "bad dreams," incorporating the awakening

A pilot trial of cladribine (2-chlorodeoxyadenosine) in remitting-relapsing multiple sclerosis

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key words: multiple sclerosis, cladribine, immunosuppression

SUMMARY

Lymphocytotoxic nucleoside analog cladribine (2-chlorodeoxyadenosine) has recently been reported to favourably alter the clinical course of chronic progressive multiple sclerosis (MS). In the present study 10 patients with the remitting-relapsing form of MS were treated with six courses of this drug (5 mg subcutaneously or 10 mg orally, once daily, repeated on five consecutive days) given once a month, followed by two additional courses at three month intervals. The patients were observed for two years after the initiation of the therapy. The treatment resulted in the reduction of lymphocyte counts to approx. 40% of the initial value at 6 months, with a trend toward recovery evident only at 24 months. Neurological status of the patients (expressed semi-quantitatively according to the EDSS scale) showed a significant improvement between 6 and 15 month of the study. The number of relapses, compared to the two-year period immediately before the treatment, remained unchanged in three patients, and was markedly reduced (almost five times on average) in the remaining seven patients. Patients who experienced the reduced relapse rate also seemed to show longer and more pronounced improvement in their neurological status.

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INTRODUCTION

According to the prevailing theory, multiple sclerosis (MS) is an autoimmune disease in which abnormalities in immune regulation lead to the lymphocyte-dependent demyelination process in the central nervous system [1,2]. The most common therapeutic approach to autoimmune diseases is based on the use of drugs producing general immunosuppression. Clinical trials with available immunosuppressants (such as cyclophosphamide, metothrexate, or cyclosporin A) evidenced at most a modest and transient benefit to MS patients [3,4]. However, until more specific treatments are discovered, general immunosuppression is considered a theoretically justified therapeutic option in this disease.

Cladribine (2-chlorodeoxyadenosine) is a purine analog with a potent and clinically useful activity against some indolent leukemias and lymphomas [5,6]. The drug displays a highly selective toxicity toward malignant lymphocytes, and normal lymphocytes are also subject to the cytotoxic effect of the drug. Cytotoxicity is mediated by cladribine phosphorylation by deoxycytidine kinase, the enzyme located predominantly in lymphoid cells [7]. Cladribine phosphates which accumulate in lymphoid cells trigger cellular events resembling to some extent the effects of irradiation, namely the accumulation of DNA strand breaks leading to programmed cell death [8].

Cladribine-induced immunosuppression is a side effect in the therapy of lymphoid malignancies. However, immunouppressive activity of the drug may be useful in the treatment of diseases of

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autoimmune etiology. Treatment with repeated doses of cladribine appeared to halt the progression of progressive multiple sclerosis [9], although a possibility of hematological toxicity of the drug (bone marrow depression) raised some concern [10]. In the present study we tested clinical efficacy of cladribine in remitting-relapsing form of MS in a two-year open-label pilot clinical trial.

MATERIAL AND METHODS

The protocol of the study was approved by the Ethical Committee of the Medical University of Lublin. Participation in the experimental trial of cladribine was offered to a limited group of multiple sclerosis patients with a definitive remitting-relapsing course of the disease, and a certain degree of neurological deficit, who were enrolled in the outpatient service of the Clinical and Research Center for Demyelinating Diseases. Clinical diagnosis of MS was additionally confirmed by brain MRI scans immediately prior to the start of the trial. Patients at relapse, or with evidence of secondary progressive course of the disease were not admitted. Further exclusion criteria were active infections, blood cytopenia of any kind, laboratory evidence of kidney or liver dysfunction, and hepatitis B antigenemia.

The study group consisted of 10 patients (eight females and two males), aged 21-51 years (median: 35 years), body weight 52-75 kg (median: 66 years). The time from the initial diagnosis of MS was 2-16 years (median: 9 years). During the two-year period immediately before entering the study the number of relapses reported by individual patients varied from 2 to 6 (3.8 per patient on average). The neurological status prior to the start of the therapy, expressed semiquantitatively in the EDSS scale [11], ranged from 1 to 6.5 (median: 4.5). The patients selected to participate in the trial were informed about the experimental drug status of cladribine, and possible side effects of therapy, and gave their written consent. Those in reproductive age were instructed to avoid pregnancy, or not to father a child, for at least two years from the beginning of the therapy.

Cladribine (2-CdA) used in the present study was synthesized and supplied free of charge by the Foundation for the Development of Diagnostics and Therapy (Warsaw, Poland). The drug was prepared as sterile solution in isotonic saline, 1 mg/ml for oral use and 2.5 mg/ml (phosphate-buffered at pH 7.4) for subcutaneous injections.

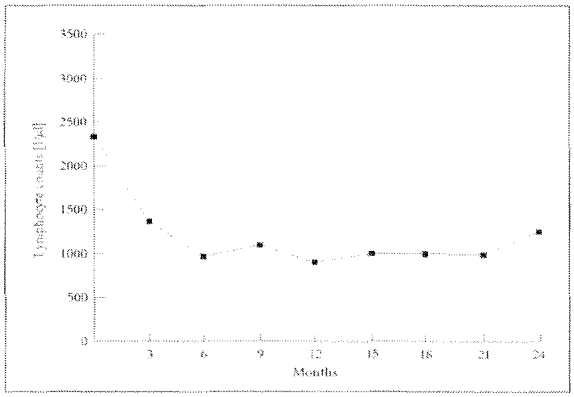
Six cladribine courses were given at monthly intervals, and two additional courses were given at 9 and 12 or 15 months. Each course consisted of five doses of the drug taken once daily on consecutive days. Cladribine was given either subcutaneously (dose 5 mg per day, six patients) or orally (dose 10 mg per day, four patients). These dosing regimens produce equivalent area under the concentration-time curve of the drug [12]. Blood counts and neurological examinations were taken at prescheduled days (preceeding the beginning of each treatment course) at monthly intervals during the first 6 months, and later every three months. The patients were instructed to maintain a close contact with supervising physicians during the study period, and to refer to them immediately in case of relapse, infection, or any other unusual event. Steroids were allowed, if severe relapse occurred.

Significance of changes in the EDSS scores were assessed by non-parametric statistical methods (Friedman ANOVA and post-hoc Wilcoxon matched pairs test), using Statistica software package.

RESULTS

Compliance of patients and tolerance of therapy was good, and hematological side effects were mild. Granulocyte counts remained relatively constant at about 4 000 per μl on average. Platelet counts dropped only slightly (to approx. 200.000 on average, and not in a single case below 100 000 per μl). Lymphocyte counts dropped from the initial count of 2 336±595 per μl (mean±S.D.) to 968±229 per μl at 6 months, and remained at approximately 1000 per μl for the next 15 months. A tendency toward normalization of lymphocyte

Figure 1. Average lymphocyte counts during and after treatment with cladribine.

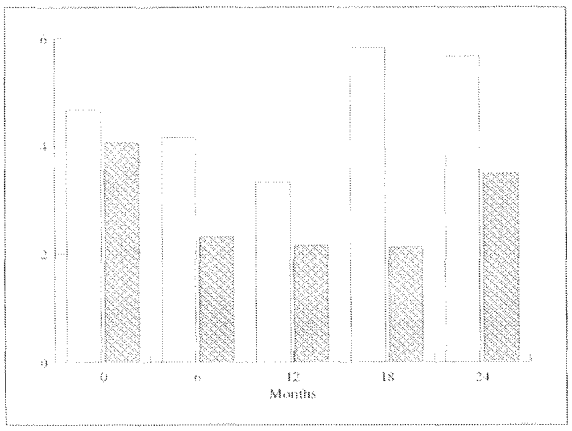


counts became evident only at the end of the 2-year observation period (fig. 1). The magnitude of lymphocyte count reduction varied among the patientswas different in particular cases, ranging from virtually no effect in one patient to a transient drop by 80% of the initial value (to less than 500 per μl) in another one. However, there was no correlation between the depth of lymphocyte nadir, or the magnitude of lymphocyte count reduction from the initial value, and the drug dose expressed per kg of body weight (normalized for oral vs. subcutaneous route of administration). Two out of four patients taking cladribine orally complained transiently of upper abdominal pain, but the association of this effect with drug intake could not be ascertained. During the study period 17 infection episodes (most of them upper respiratory tract or urinary tract infections) occurred in the whole group (including eight in a single patient). All infections responded to standard antibiotic therapy, creating no significant threat.

Table 1. Average EDSS scores during and after the treatment.

Month after the initiation of treatment	EDSS (mean±SD)	p
0	4.3±1.8	–
3	3.4±1.5	0.01
6	2.9±1.7	0.02
9	2.7±1.9	0.02
12	2.6±1.6	0.01
15	2.4±1.7	0.02
18	3.3±2.4	0.26
21	3.4±2.5	0.14
24	4.3±2.0	0.34

Figure 2. Average EDSS scores „responders" (filled bars) and „non-responders" (open bars).



Analysis of the data for the whole group revealed that EDSS scores were significantly reduced during the treatment compared to the initial values (Friedman ANOVA $\chi^2(N=6, df=8)=18.07$, $p<0.02$, and that the decreases observed at the third to fifteenth month were significant by Wilcoxon matched pairs test (Table 1). The total number of relapses reported by the patients during the two years immediately preceeding the initiation of the treatment was 38, whereas it was only 15 during the two-year study period.

Further inspection of the data revealed that, from the point of view of the apparent efficacy of the treatment, the group under study can be divided into two sub-groups, the 'responsders' and the 'non-responders'. The 'responders' sub-group consisted of seven patients who reported a total of 29 relapses during the two preceeding years (average relapse rate 2.07 per patient per year), and a total of 6 relapses during the two-year study period (average relapse rate 0.43 per patient per year). Two of the responders reported no relapses for the entire two-year period of treatment and post-treatment follow-up, whereas during the two preceeding years one of them experienced three, and the other one four relapses. The 'non-responders' subgroup consisted of three patients whose relapse rate was the same during the two-year periods prior to and after the initiation of the therapy (total of 9 relapses, average relapse rate 1.5 per patient per year). Averaged EDSS scores for 'responders' and 'non-responders' are shown in Fig. 2. One patient who did not respond to treatment displayed only a slight and transient reduction in lymphocyte count during the treatment, but the other two showed the reduction of approximately the same magnitude as in some of the 'responders'. The average body weight was the same in both sub-groups.

DISCUSSION

Clinical trials of general immunosuppressants in multiple sclerosis produced disappointinging results. The reason may be that the depth of immunosuppression required for successful modification of the natural history of MS cannot be achieved with 'conventional' immunosuppressive drugs because of systemic side effects. Indeed, it has been shown in a double-blind placebo-controlled trial with cyclosporin A in relapsing-remit-

ting MS [13] that beneficial effect of the therapy (reduction in relapse rate) could be achieved only when high (and toxic) doses of the drug were given, so that severe concomitant side effects (hypertension, renal insufficiency and anemia) precluded such treatment.

The present trial was performed with a small number of patients, and was not placebo-controlled. Its results concerning the efficiacy of the treatment shall, therefore, be interpreted with great caution. Nevertheless, we consider them potentially important. The therapy appeared to be effective in seven patients who reported a very marked (almost five-fold on average) reduction in the relapse rate during the 2 years after the initiation of the treatment, while it seemed ineffective in the remaining three. The 'responders' showed also a somewhat more pronounced improvement in their neurological status as measured by EDSS scores. We were unable to identify any factor which would differentiate between the 'responders' and the 'non-responders'. There was no indication that a 'good response' is related to the actual cladribine dose per body weight. Although the reduction of lymphocyte count was only minute and transient in one 'non-responder', in the other two the lymphocyte drops were within the range of reductions observed in the 'responders'.

When side effects of the therapy are considered, cladribine favourably compares with other immunosuppressants. In the treatment of lymphoid malignancies, besides an increased incidence of infections, the only relatively frequent side effect is thrombocytopenia (which, at least in some cases, may be related to the marrow involvement in the disease process), but there is virtually no non-hematologic toxicity at the doses up to 0.1 mg/kg daily i.v. for five to seven days days [5,6]. The limited toxicity of cladribine is attributed to the confinement of the drug-activating enzyme (deoxycytidine kinase) to the lymphoid cells. Although dangerous marrow depression were reported in some multiple sclerosis patients treated with cladribineby Beutler et al [10], our experience (extended already to a larger group of patients [14]) indicates that in this clinical setting a two- to three-fold reduction of blood lymphocyte count can be achieved by the drug given subcutaneously without clinically significant hematological side effects.

There are some aspects of cladribine pharmacokinetics and pharmacodynamics which may be responsible for its beneficial activity in MS. The drug is able to cross the blood-brain barrier resulting in CSF: plasma ratio of 25% [15,16], so that it may exert some toxicity also toward lymphocyte clones inhabitating the CNS. It displays, at least in the in vitro conditions, immunosuppressive effects not related to the simple reduction of lymphocyte counts: it inhibits T and B cell activation and the response of T cells to co-stimulation by proteins of the extracellular matrix [17,18]. Its property of stimulating the activity of the NK cells in vitro [19] and restoring their impaired activity in vivo (observed in leukemic patients by Lauria et al [20]) may also be of some significance, because in relapsing-remitting MS new MRI-visible lesions were reported to appear only during the periods of reduction of NK functional activity [21].

CONCLUSIONS

In patients with remitting-relapsing multiple sclerosis treatment with cladribine decreases lymphocyte counts in peripheral blood, to 1/3 of the initial values on average. The relapse rate in some (but not all) patients is impressively decreased. Side effects of the therapy are mild. The reason why some patients do not respond to the treatment remains to be identified. Along with clinical evaluation in MS, the detailed pattern of cladribine-induced immunosuppression in clinical conditions deserves further study.

REFERENCES:

1. *French-Constant C: Pathogenesis of multiple sclerosis. Lancet, 1994; 343: 271-275*
2. *Hafler DA, Weiner HL (1989) MS: a CNS and systemic autoimmune disease. Immunol Today, 1989; 10: 104-107*
3. *Hughes R: Immunotherapy for multiple sclerosis (Editorial). J Neurol Neurosurg Psychiat, 1994; 57: 3-6*
4. *Noseworthy JH: Immunosuppressive therapy in multiple sclerosis: pros and cons. Int MSJ, 1994; 1: 79-89*
5. *Beutler E: Cladribine (2-chlorodeoxyadenosine). Lancet, 1992, 340: 952-956*
6. *Saven A, Piro LD: 2-Chlorodeoxyadenosine: a newer purine analog active in the treatment of indolent lymphoid malignancies. Ann Intern Med, 1994, 120: 784-791*
7. *Carson DA, Wasson DB, Taetle R, Yu A: Specific toxicity of 2-chlorodeoxyadenosine towards resting and proliferating human lymphocytes. Blood, 1983; 3: 737-743*
8. *Carrera CJ, Piro LD, Saven A, Beutler E, Terai C, Carson DA: 2-Chlorodeoxy- adenosine chemotherapy triggers programmed cell death in normal and malignant lymphocytes. In: Purine and Pyrimidine Metabolism in Man. (Harkness RA et al, eds), Plenum Press, New York, 1991, pp. 15-18*
9. *Beutler E, Sipe JC, Romine JS, Koziol JA, McMillan R, Zyroff J: The treatment of chronic progressive multiple sclerosis with cladribine. Proc Natl Acad Sci USA, 1996, 76: 2436-2433*
10. *Beutler E, Koziol JA, McMillan R, Sipe JC, Romine JS, Carrera CJ: Marrow suppression produced by repeated doses of cladribine. Acta Haematol, 1994; 91: 10-15*

11. Kurtzke JF: Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology, 1983; 33: 1444-1452

12. Liliemark J, Albertioni F, Hassan M, Juliusson G: On the bioavailability of oral and subcutaneous 2-chloro-2'-deoxyadenosine in humans: alternative routes of administration. J Clin Oncol, 1991; 10: 1514-1518

13. Rudge P, Koetsier JC, Mertin T, Mispelblombeyer JO, van Walbeck HK, Clifford Jones R, Harrison J, Robinson K, Mellein B, Poole T, Stokvis JCJM, Timonen P: Randomised double blind controlled trial of cyclosporin in multiple sclerosis. J Neurol Neurosurg Psychiat, 1989; 52: 559-565

14. Stelmasiak Z, Solski J, Jakubowska B, Nowicki J, Grieb P: (1995) Safety of cladribine treatment in multiple sclerosis (Abstract). J Neuroimmunol, 1995, Suppl. 1, 17

15. Kearns CM, Blakley RL, Santana VM, Crom WR: Pharmacokinetics of cladribine (2-chlorodeoxyadenosine) in children with acute leukemia. Cancer Res, 1994; 54: 1235-1239

16. Liliemark J, Juliusson G: On the pharmacokinetics of 2-chloro-2'-deoxyadenosine (CdA) in cerebrospinal fluid (CSF). (Abstract) Blood, 1992; 80 (Suppl. 1), 471a

17. Górski A, Grieb P, Korczak-Kowalska G, Wierzbicki P, Stępień-Sopniewska B: Cladribine: an inhibitor of human B and T cell activation in vitro. Immunopharmacology, 1993; 26: 197-202

18. Górski A, Grieb P, Makuła J, Stępień-Sopniewska B, Mrowiec M, Nowaczyk M: 2-Chloro-2'-deoxyadenosine - a novel immunosuppressive agent. Transplantation, 1993; 56: 1253-1257

19. Priebe T, Kandil O, Nakic M, Fang Pan B, Nelson JA: Selective modulation of antibody response and natural killer cell activity by purine nucleoside analogues. Cancer Res, 1988; 48: 4799-4803

20. Lauria F, Rondelli LF, Raspadori D, Benfenati D, Tura S: Rapid restoration of natural killer activity following treatment with 2-chlorodeoxyadenosine in 22 patients with hairy cell leukemia. Eur J Hematol, 1994; 52: 16-20

21. Kastnikoff LF, Morgan N, Zecchini D, Satoh J, Paty DW: (1995) Correlation of natural killer (NK) cell functional activity and phenotype with disease activity determined clinically and by serial MRIs. Effects of interferon-(-1b (Abstract). J Neuroimmunol, 1995, Suppl. 1: 64

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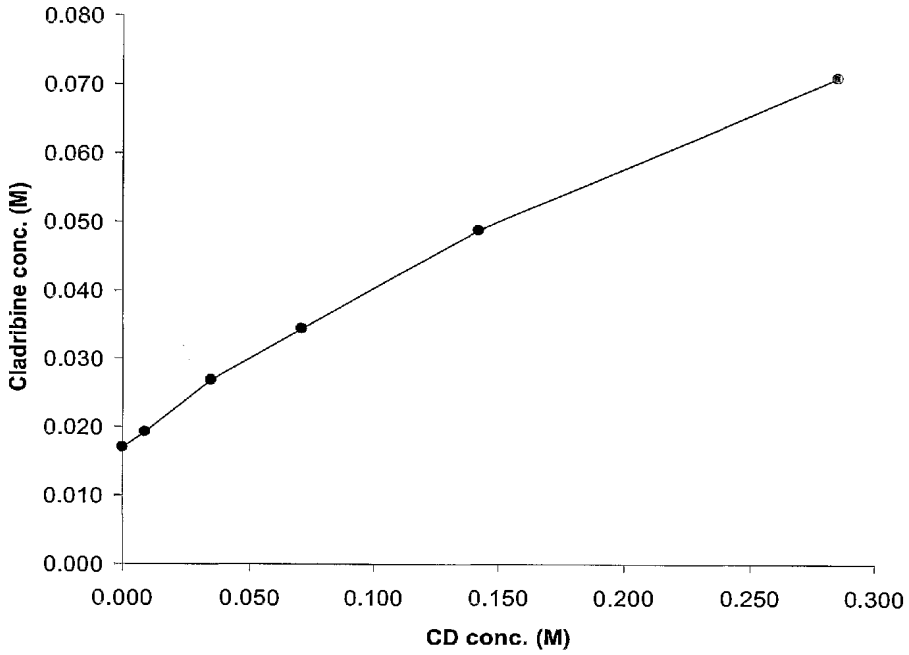
(54) Title: ORAL FORMULATIONS OF CLADRIBINE



(57) Abstract: ABSTRACT OF THE DISCLOSURE Provided are compositions of cladribine and cyclodextrin which are especially suited for the oral administration of cladribine.

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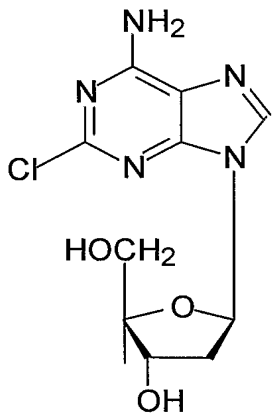
ORAL FORMULATIONS OF CLADRIBINE

FIELD OF THE INVENTION

The invention relates to a composition comprising a complex cladribine-cyclodextrin complex formulated into a solid oral dosage form and to a method for enhancing the oral bioavailability of cladribine.

BACKGROUND OF THE INVENTION

Cladribine, which is an acid-labile drug, has the chemical structure as set forth below:



It is also known as 2-chloro-2′-deoxyadenosine or 2-CdA. Cladribine exists as a white, nonhydroscopic, crystalline powder, consisting of individual crystals and of crystalline aggregates.

Cladribine is an antimetabolite which has use in the treatment of lymphoproliferative disorders. It has been used to treat experimental leukemias such as L1210 and clinically for hairy cell leukemia and chronic lymphocytic leukemia as well as Waldenstrom's macroglobulinaemia. It has

also been used as an immunosuppressive agent and as a modality for the treatment of a variety of autoimmune conditions including rheumatoid arthritis, inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis) and multiple sclerosis (see *e.g.*, J. Liliemark, *Clin. Parmacokinet*, 32(2): 120-131, 1997). It has also been investigated, either experimentally or clinically in, for example, lymphomas, Langerhan's cell histiocytosis, lupus erythematosus, chronic plaque psoriasis, Sezary syndrome, Bing-Neel syndrome, recurrent glioma, and solid tumors.

Oral delivery of drugs is often preferred to parenteral delivery for a variety of reasons, foremost patient compliance, or for cost or therapeutic considerations. Patient compliance is enhanced insofar as oral dosage forms alleviate repeated health care provider visits, or the discomfort of injections or prolonged infusion times associated with some active drugs. At a time of escalating health care costs, the reduced costs associated with oral administration versus parenteral administration costs gain importance. The cost of parenteral administration is much higher due to the requirement that a health care professional administer the cladribine in the health care provider setting, which also includes all attendant costs associated with such administration. Furthermore, in certain instances, therapeutic considerations such as the need for a slow release of cladribine over a prolonged period of time may be practically met only by oral or transmucosal delivery.

However, to date the oral delivery of cladribine has been plagued by low bioavailability (see, *e.g.*, J. Liliemark *et al., J. Clin. Oncol.*, 10(10): 1514-1518, 1992), and suboptimal interpatient variation (see, *e.g.*, J. Liliemark, *Clin. Pharmacokinet*, 32 (2): 120-131, 1997). See also, A. Tarasuik, *et al.* reporting poor absorption and pH dependent lability (*Arch. Immunol. et Therapiae Exper.*, 42: 13-15, 1994).

Cyclodextrins are cyclic oligosaccharides composed of cyclic α-(1→4) linked D-glucopyranose units. Cyclodextrins with six to eight units have

been named α-, β- and γ-cyclodextrin, respectively. The number of units determines the size of the cone-shaped cavity which characterizes cyclodextrins and into which drugs may be included to form stable complexes. A number of derivatives of α-, β- and γ-cyclodextrin are known in which one or more hydroxyl groups is/are replaced with ether groups or other radicals. These compounds are thus known complexing agents and have been previously used in the pharmaceutical field to form inclusion complexes with water-insoluble drugs and to thus solubilize them in aqueous media.

Recently, Schultz *et al.*, in U.S. Patent No. 6,194,395 B1, have described complexing and solubilizing cladribine with cyclodextrin. The Schultz *et al.* patent primarily addresses the problems inherent in previously described aqueous formulations of cladribine, particularly for subcutaneous and intramuscular injection. Schultz *et al.* have found that cladribine is not only significantly more soluble in aqueous media when formulated with cyclodextrin, but also is more stable against acid-catalyzed hydrolysis when combined with cyclodextrin. The latter finding is taught to be of particular benefit in the formulation of solid oral dosage forms, where the compound would normally undergo hydrolysis in the acid pH of the stomach contents. Schultz *et al.* do not appear to have described any actual work in connection with solid oral dosage forms. In fact, they describe only one method of preparing the solid dosage form, which is a melt extrusion process, in which the cladribine and cyclodextrin are mixed with other optional additives and then heated until melting occurs. Furthermore, the broad dosage ranges of 1 mg to 15 mg of cladribine and 100 mg to 500 mg of cyclodextrin listed in the patent suggest no criticality to the particular amount of cyclodextrin to be present with a given amount of cladribine in a solid oral dosage form. Indeed, these dosage ranges include many combinations which may be suitable as mixtures but not for complex formation. For example, a ratio of 1 mg of cladribine to 500 mg of cyclodextrin contains too much cyclodextrin, so

that the drug would not readily leave the complex and achieve its therapeutic function. On the other hand, 15 mg of cladribine and only 100 mg of cyclodextrin would not be enough to complex that amount of cladribine.

The Schultz *et al.* patent does suggest improving the stability of cladribine in oral dosage forms by combining/complexing it with cyclodextrin, but does not suggest improving the drug's oral bioavailability by such means; in fact, the patent does not describe or suggest a method for enhancing or maximizing the bioavailability of cladribine from a solid oral dosage form of cladribine and cyclodextrin, or a composition specially designed to do so.

Many workers have studied the solubility of specific drugs in water containing various concentrations of selected cyclodextrins in order to demonstrate that increasing concentrations of cyclodextrins increase the solubility of the drugs at selected temperatures and pH levels, as for example reported in the Schultz *et al.* patent. Phase solubility studies have also been performed by various workers in order to elucidate the nature of the complex formation, for example, whether the cyclodextrin and drug form a 1:1 complex or a 1:2 complex; see, for example, Harada *et al.* U.S. Patent No. 4,497,803, relating to inclusion complexes of lankacidin-group antibiotics with cyclodextrin, and Shinoda *et al.* U.S. Patent No. 4,478,995, relating to a complex of an acid addition salt of (2′-benzyloxycarbonyl)phenyl trans-4-guanidinomethylcyclohexanecarboxylate with a cyclodextrin.

While Schultz *et al.* teach that a cladribine-cyclodextrin complex improves the water solubility and acid stability of cladribine, the art does not suggest how to maximize or enhance the benefits of the complexation in terms of bioavailability and interpatient variation when the complex is to be administered in a solid oral dosage form.

SUMMARY OF THE INVENTION

It has now been found that amorphous cyclodextrins can be combined with cladribine to form a particularly advantageous product which can be incorporated into a solid oral dosage form. This product is a complex cladribine-cyclodextrin complex, and the solid oral dosage form containing it improves oral bioavailability and/or achieves lower interpatient and/or intrapatient variation of the drug.

The present invention provides a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, and a pharmaceutical composition comprising said complex, formulated into a solid oral dosage form. Thus, the cyclodextrin itself is amorphous, the inclusion complex with cladribine is amorphous (and is preferably saturated with cladribine) and the free cladribine which forms the non-inclusion complex is amorphous.

The invention also provides a method for increasing or enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof, a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form which maximizes the amount of cladribine in the inclusion and non-inclusion complexes.

The invention further provides for treatment of conditions responsive to administration of cladribine in mammals by administering thereto the composition of the invention. Use of cladribine in the preparation of the pharmaceutical compositions of the invention for administration to treat

cladribine-responsive conditions and for enhancing the oral bioavailability of cladribine is also provided.

Still further, the invention provides a process for the preparation of a complex cladribine-cyclodextrin complex which comprises the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature; and

(iii) lyophilizing the cooled solution to afford an amorphous product.

In yet a further aspect the invention provides a pharmaceutical composition obtainable by a process comprising the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature;

(iii) lyophilizing the cooled solution to afford an amorphous product; and

(iv) formulating the amorphous product into a solid oral dosage form.

BRIEF DESCRIPTION OF THE DRAWING

A more complete appreciation of the invention and its many attendant advantages will be readily understood by reference to the following detailed description and the accompanying drawing, wherein the sole Figure is a graphical representation of the results of a phase solubility study where various molar concentrations of hydroxypropyl-β-cyclodextrin (HPβCD) are plotted against various cladribine molar concentrations, with (●) representing

the data points obtained for complexation under conditions specified in EXAMPLE 2 below.

DETAILED DESCRIPTION OF THE INVENTION

Throughout the instant specification and claims, the following definitions and general statements are applicable.

The patents, published applications, and scientific literature referred to herein establish the knowledge of those with skill in the art and are hereby incorporated by reference in their entirety to the same extent as if each was specifically and individually indicated to be incorporated by reference. Any conflict between any reference cited herein and the specific teachings of this specification shall be resolved in favor of the latter. Likewise, any conflict between an art-understood definition of a word or phrase and a definition of the word or phrase as specifically taught in this specification shall be resolved in favor of the latter.

The term "inclusion complex" as used herein refers to a complex of cladribine with the selected cyclodextrin wherein the hydrophobic portion of the cladribine molecule (the nitrogen-containing ring system) is inserted into the hydrophobic cavity of the cyclodextrin molecule. This is often referred to simply as a cyclodextrin complex of the drug.

The term "non-inclusion complex" refers to a complex which is not an inclusion complex; rather than the hydrophobic portion of cladribine being inserted in the cyclodextrin cavity, the non-inclusion complex is formed primarily by hydrogen-bonding of the hydroxyls and amino group on "free" cladribine, (*i.e.* cladribine not in the inclusion complex) to the hydroxyls on the exterior of the cyclodextrin torus (*e.g.* in the case of hydroxypropyl-β-cyclodextrin, hydroxypropyl and hydroxyl groups on the glucose rings). This is a more loosely-held association than an inclusion complex.

As used herein, whether in a transitional phrase or in the body of a claim, the terms "comprise(s)" and "comprising" are to be interpreted as having an open-ended meaning. That is, the terms are to be interpreted synonymously with the phrases "having at least" or "including at least". When used in the context of a process, the term "comprising" means that the process includes at least the recited steps, but may include additional steps. When used in the context of a composition, the term "comprising" means that the composition includes at least the recited features or components, but may also include additional features or components.

The terms "consists essentially of" or "consisting essentially of" have a partially closed meaning, that is, they do not permit inclusion of steps or features or components which would substantially change the essential characteristics of a process or composition; for example, steps or features or components which would significantly interfere with the desired properties of the compositions described herein, *i.e.,* the process or composition is limited to the specified steps or materials and those which do not materially affect the basic and novel characteristics of the invention. The basic and novel features herein are the provision of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form, so as to provide improved bioavailability and/or lower interpatient and/or intrapatient variation following administration. Essential to the invention is the combination of the amorphous nature of the starting cyclodextrin, and the level of water solubility exhibited by cladribine (about 5 mg/ml at room temperature), and consequently its capability for hydrogen bonding, which can be taken advantage of under particular conditions described hereinafter, and which afford a special amorphous mixture uniquely well-suited for optimizing the oral bioavailability of cladribine.

The terms "consists of" and "consists" are closed terminology and allow only for the inclusion of the recited steps or features or components.

As used herein, the singular forms "a," "an" and "the" specifically also encompass the plural forms of the terms to which they refer, unless the content clearly dictates otherwise.

The term "about" is used herein to means approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" or "approximately" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

The term "amorphous" is used herein to refer to a noncrystalline solid. The cyclodextrins encompassed herein themselves are amorphous because they are each composed of a multitude of individual isomers, and their complexes with cladribine are also amorphous. Further, conditions for complexation can be selected (elevated temperature and prolonged complexation times, as described hereinafter) so that a supersaturated cladribine solution will be formed. When cooled, because of the amorphous nature of the complex and the cyclodextrin, some excess free cladribine does not precipitate but rather is trapped in amorphous form in intimate admixture with the (preferably saturated) amorphous cladribine-cyclodextrin inclusion complex. This excess cladribine forms a loosely-held association, or non-inclusion complex, with the cyclodextrin through hydrogen bonding. This, then, further increases the amount of cladribine in the product; this additional cladribine, because it is amorphous and also because it is in intimate admixture with the amorphous inclusion complex, is expected to be somewhat protected from degradation by stomach acid (although it may not be as protected as the cladribine which is in the form of the inclusion complex).

The term "saturated" when used in conjunction with a complex of cladribine in amorphous cyclodextrin means that the complex is saturated with cladribine, that is, the complex contains the maximum amount of cladribine which can be complexed (by means of both inclusion and non-inclusion complexes) with a given amount of cyclodextrin under the conditions of complexation used. A phase solubility study can be used to provide this information, as described in more detail hereinafter. (Conditions for the complexation are also described in more detail below.) Alternatively, a saturated complex may be arrived at empirically by simply adding cladribine to an aqueous solution of the selected cyclodextrin until no more cladribine goes into solution; ultimately, excess cladribine, if any, is removed (by filtration or centrifugation) and the solution lyophilized to provide the dry saturated complex.

The expression "substantially', as in "substantially free" means within 20% of the exact calculated amount, preferably within 10%, most preferably within 5%.

The term "interpatient variability" refers to variation among patients to which a drug is administered. The term "intrapatient variability" refers to variation experienced by a single patient when dosed at different times.

As used herein, the recitation of a numerical range for a variable is intended to convey that the invention may be practiced with the variable equal to any of the values within that range. Thus, for a variable which is inherently discrete, the variable can be equal to any integer value of the numerical range, including the end-points of the range. Similarly, for a variable which is inherently continuous, the variable can be equal to any real value of the numerical range, including the end-points of the range. As an example, a variable which is described as having values between 0 and 2, can be 0, 1 or 2 for variables which are inherently discrete, and can be 0.0,

0.1, 0.01, 0.001, or any other real value for variables which are inherently continuous.

In the specification and claims, the singular forms include plural referents unless the context clearly dictates otherwise. As used herein, unless specifically indicated otherwise, the word "or" is used in the "inclusive" sense of "and/or" and not the "exclusive" sense of "either/or."

Technical and scientific terms used herein have the meaning commonly understood by one of skill in the art to which the present invention pertains, unless otherwise defined. Reference is made herein to various methodologies and materials known to those of skill in the art. Standard reference works setting forth the general principles of pharmacology include Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 10th Ed., McGraw Hill Companies Inc., New York (2001).

Reference is made hereinafter in detail to specific embodiments of the invention. While the invention will be described in conjunction with these specific embodiments, it will be understood that it is not intended to limit the invention to such specific embodiments. On the contrary, it is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims. In the following description, numerous specific details are set forth in order to provided a thorough understanding of the present invention. The present invention may be practiced without some or all of these specific details. In other instances, well-known process operations have not been described in detail, in order not to unnecessarily obscure the present invention.

There is provided by the present invention compositions, as well as methods of making and of using pharmaceutical compositions, useful to achieve desirable pharmacokinetic properties. Such compositions stem from the discovery that solutions of cyclodextrin and cladribine in which cladribine is in a high thermodynamic state, when presented to the gastric mucosa

through which they are absorbed are associated with improved cladribine absorption, as reflected by higher bioavailability and/or lower interpatient variation.

It is postulated, without wishing to so limit the invention, that upon dissolution (*e.g.,* by contact with a fluid, such as a bodily fluid), dry compositions according to the invention form a locally saturated cladribine solution in which cladribine is in the state of highest thermodynamic activity (HTA), thus favoring absorption. Cladribine has a fairly low, although not insignificant, intrinsic aqueous solubility; it is in fact somewhat water soluble. The free cladribine formed from dissociation of the inclusion and non-inclusion complexes in a saturated aqueous solution seeks a more stable activity level by being absorbed through the gastric mucosa.

In view of the foregoing, it is apparent that to produce optimal pharmaceutical compositions, in a solid oral dosage form, these dosage forms should be formulated to release a localized saturated cladribine solution, upon contact of the solid dosage forms with body fluid at the mucosa, in which cladribine is in its HTA state. To provide such a localized saturated solution *in vivo*, it is important to first identify the optimal ratio of cladribine to amorphous cyclodextrin, which ratio is referred to herein as the HTA ratio, to be used in the solid dosage form.

The HTA ratio is empirically determined and is identified as the ratio of cladribine to amorphous cyclodextrin which corresponds to the maximum amount of cladribine that can be complexed with a given amount of the cyclodextrin. The HTA ratio may be determined using an empirical method such as a phase solubility study to determine the saturation concentration of cladribine that can be solubilized with different concentrations of amorphous cyclodextrin solutions. Hence, the method identifies the concentrations at which a saturated cladribine-cyclodextrin complex is formed. It is noted that the molar ratio represented by a point on the phase solubility graph shows

how many moles of amorphous cyclodextrin are the minimum needed to maintain the drug in the complex, under given conditions; this may then be converted to a weight ratio. For example, if a phase solubility diagram shows that 9 moles of a given cyclodextrin are needed to maintain the cladribine in a saturated complex, then multiplying the number of moles of cladribine by its molecular weight and multiplying the number of moles of the selected cyclodextrin by its molecular weight, one can arrive at the ratio of the products as an appropriate optimized weight ratio. A phase solubility study also provides information about the nature of the cladribine-cyclodextrin inclusion complex formed, for example whether the inclusion complex is a 1:1 complex (1 molecule of drug complexed with 1 molecule of cyclodextrin) or a 1:2 complex (1 molecule of drug complexed with 2 molecules of cyclodextrin).

In accordance with the present invention, one can start using either the selected amorphous cyclodextrin, such as hydroxypropyl-β-cyclodextrin (HPβCD) or hydroxypropyl-γ-cyclodextrin, or cladribine as the fixed variable to which an excess of the other is added to identify various solubility data points (indicating saturated cladribine-cyclodextrin complexes) and draw the resultant line. Typically, cladribine is added to an aqueous solution having a known concentration of amorphous cyclextrin under conditions empirically found to promote complex formation. Generally, the complexation is conducted with heating, for example at about 45 to about 60°C for a significant period of time, *e.g.*, at least 6-9 hours; it is believed that even better results can be obtained by heating at up to about 80°C for up to 24 hours. Excess precipitated cladribine is then removed and the cladribine concentration is subsequently measured. This concentration represents the amount of cladribine solubilized for a given amorphous cyclodextrin concentration. This process is repeated for a different known concentration of cyclodextrin until several data points are obtained. Each data point represents the concentration of the cladribine dissolved in a known

concentration of the selected amorphous cyclodextrin. The data points are then plotted to show the concentration of cladribine against the various cyclodextrin concentrations used. The graph is a phase solubility diagram which can be used to determine the amount of cladribine for any specific concentration of cyclodextrin used to form the solution under a given set of complexation conditions. It will be appreciated that the aqueous solubility of cladribine is about 5 mg/ml at room temperature and would be higher at elevated temperature. Consequently, the data points correspond to the amount of cladribine dissolved in aqueous HPβCD or other amorphous cyclodextrin under the selected conditions; when later lyophilized, the solution yields a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex. If equilibrium conditions are reached during the complexation, the amorphous cladribine-cyclodextrin complex will be saturated with cladribine.

One of skill in the art will appreciate that concentrations at which saturated complexes of cladribine with amorphous cyclodextrins are formed (and thus HTA ratios as well) may be identified by a variety of alternative methodologies. Accordingly, any method known in the field suitable to identify these concentrations is within the scope of the invention.

It has been discovered that desirable pharmacological properties (improved bioavailability and/or coefficient of variation as compared to traditional approaches) are associated with mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin.

Using intrinsically amorphous cyclodextrins, for example hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated cyclodextrins, and the like, with cladribine, which is a somewhat water soluble compound (capable of H-bonding through its free hydroxyl and

amino groups), the cladribine provides increased solubility in solutions of these cyclodextrins. Not only is there increased water solubility but also H-bonded association of the cladribine with the cyclodextrin, separately from the actual inclusion complexed material.

One of skill in the art will appreciate that the phase solubility diagram for each given starting concentration ratio represents the starting point of one's investigation on the basis of which variables (reactants' concentrations, temperature and time) may be altered to promote inclusion complex and non-inclusion complex associations favoring a higher or lower proportion of either type of association in the final product. Departure from the ratio of cladribine to cyclodextrin, the temperature and/or the dilution empirically found to promote equilibrium towards complex formation is then analyzed to promote the formation of mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin in various proportions according to the invention.

Thus, for example, by starting with more diluted cyclodextrin (*i.e.*, larger water volumes than that used for solubility plot analysis) logically will accommodate more cladribine in solution sequestering more of the same from complex formation. Upon evaporation, some of the solubilized cladribine will tend to associate with cyclodextrin in a non-inclusion complex fashion. By altering the initial dilution, one may shift equilibrium towards inclusion complex or non-inclusion complex formation. Similarly, by increasing complexation temperature, the water solubility of cladribine may be increased while decreasing the stability of inclusion complexes, thus promoting non-inclusion complexes. Thus, by altering complexation temperature, one may shift equilibrium towards inclusion complex or non-inclusion complex formation. Finally, complexation time may be altered to favor the formation of mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin according to the invention.

As exemplified hereinafter, it is possible to maximize the cladribine in solid amorphous mixtures, by forcing additional cladribine into solution (using more dilute solutions of cyclodextrin, higher temperatures and longer complexation times, as indicated above). When the solution is cooled off, the extensively amorphous nature of these cyclodextrins does not allow crystallization of an excess amount of cladribine beyond that which forms an inclusion complex with the cyclodextrin; and upon freeze-drying/lyophilization, one obtains an amorphous mixture of cladribine-cyclodextrin inclusion complex (which is amorphous) and amorphous free cladribine, loosely associated with uncomplexed cyclodextrin (and even with complexed cyclodextrin) by hydrogen-bonding, that is, the non-inclusion complex.

As shown in the EXAMPLES, this may be done by maximizing solubilization by elevating the temperature (for example, to about 50°to 80°C), and stirring for many hours (up to 24 hours) before freeze-drying. The weight/weight ratios obtained were about 1:14 and 1:11. The apparent optimum weight/weight ratio under these exemplified conditions is the higher of these, or about 1:14 of cladribine: cyclodextrin. If too much excess caldribine is added to the complexation medium, then crystallization of some of the cladribine takes place, which would in turn result in some crystalline cladribine in the product; this undesired excess cladribine is not in solution and is not H-bonded to the amorphous cyclodextrin and lowers the weight ratio. Therefore, it is desirable to carefully control the amount of excess cladribine beyond that which will form the inclusion complex to only the amount which will dissolve in the solution. The desired amorphous mixture of amorphous inclusion complex and amorphous free cladribine can be termed a "complex cladribine-cyclodextrin complex," which includes both inclusion and non-inclusion/H-bonded complexes. The inclusion complex is a complex of cladribine inserted into the hydrophobic cavity of the selected amorphous cyclodextrin, while the non-inclusion/H-bonded complex is

amorphous free cladribine loosely hydrogen-bonded to the cyclodextrin. It is estimated that about two-thirds (60 to 70%) of the cladribine will be in the non-inclusion complex, with the remaining one third (30 to 40%) being in the inclusion complex when the product is obtained as exemplified hereinbelow (17% HPβCD solution, 45 to 50°C complexation temperature for about 9 hours); by increasing the percentage of cyclodextrin used and/or manipulating the temperature, products can be readily obtained in which a much greater proportion of the amorphous mixture is in the form of the inclusion complex. In the case of a representative amorphous cyclodextrin, hydroxypropyl-β-cyclodextrin (HPβCD) a cladribine:cyclodextrin weight ratio of from about 1:10 to about 1:16 is appropriate for the exemplified conditions; the ratio is expected to be the same for hydroxypropyl-γ-cyclodextrin under those conditions. The material obtained is characterized by rapid dissolution of the cladribine in aqueous media.

Freeze-drying, also known as lyophilization, comprises three basic stages: first a freezing stage, then a primary drying stage and finally a secondary drying stage. EXAMPLE 2 below provides details of lyophilization as conducted on the batches described therein. This procedure can be further optimized by following the principles described by Xiaolin (Charlie) Tang and Michael J. Pikal in *Pharmaceutical Research*, Vol. 21, No. 2, February 2004, 191-200, incorporated by reference herein in its entirety and relied upon.

The above-described method requires amorphous cyclodextrins rather than originally crystalline cyclodextrins which have relatively low water solubilities, such as α-, β- or γ-cyclodextrin, 2,6-dimethyl-β-cyclodextrin and the like, because these cyclodextrins would allow crystallization of cladribine in excess of that forming an inclusion complex and therefore would not afford the desired amorphous mixture. The method also would not be useful if cladribine were highly hydrophobic/lipophilic, because in such a situation the drug would not have intrinsic aqueous solubility/H-bonding capability and

could not provide the unique mixture obtained herein. However, in point of fact, cladribine has an aqueous solubility of 5 mg/ml at room temperature, thus a significant amount of the drug will be simply soluble in the water phase especially at higher than room temperature; also, as in the case of HPβCD, for example, some of the cladribine will be associated by hydrogen-bonding to the 2-hydroxypropyl and free glucose-OH groups in the cyclodextrin via the two hydroxy functions found in the deoxyadenosine moiety of the cladribine.

The cyclodextrins within the scope of this invention are amorphous derivatives of the natural cyclodextrins α-, β- or γ-cyclodextrin wherein one or more of the hydroxy groups are substituted, for example, by alkyl, hydroxyalkyl, carboxyalkyl, alkylcarbonyl, carboxyalkoxyalkyl, alkylcarbonyloxyalkyl, alkoxycarbonylalkyl or hydroxy-(mono or polyalkoxy)alkyl groups; and wherein each alkyl or alkylene moiety preferably contains up to six carbons. Although commonly referred to as a single entity, an amorphous cyclodextrin is actually a mixture of many different entities, since the substituent groups can be located on various hydroxyls of the basic cyclodextrin structure. This in turn results in the amorphous nature of these cyclodextrins, which is indeed well-known. Moreover, these cyclodextrins can be obtained in varying degrees of substitution, for example from 1 to 14, preferably from 4 to 7; the degree of substitution is the approximate average number of substituent groups on the cyclodextrin molecule, for example, the approximate number of hydroxypropyl groups in the case of the hydroxpropyl-β-cyclodextrin molecule, and all such variations are within the ambit of this invention. Substituted amorphous cyclodextrins which can be used in the invention include polyethers, for example, as described in U.S. Patent No. 3,459,731. Further examples of substituted cyclodextrins include ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is replaced by C_{1-6}alkyl, hydroxy-C_{1-6}alkyl, carboxy-C_{1-6}alkyl or C_{1-6}alkyloxycarbonyl-

C_{1-6}alkyl groups or mixed ethers thereof. In particular, such substituted cyclodextrins are ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is replaced by C_{1-3}alkyl, hydroxy-C_{2-4}alkyl or carboxy-C_{1-2}alkyl or more particularly by methyl, ethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, carboxymethyl or carboxyethyl. The term "C_{1-6}alkyl" is meant to include straight and branched saturated hydrocarbon radicals, having from 1 to 6 carbon atoms such as methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, butyl, pentyl, hexyl and the like. Other cyclodextrins contemplated for use herein included glucosyl-β-cyclodextrin and maltosyl-β-cyclodextrin. Of particular utility in the present invention are randomly methylated β-cyclodextrin and polyethers such as hydroxypropyl-β-cyclodextrin, hydroxyethyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, and hydroxyethyl-γ-cyclodextrin, as well as sulfobutyl ethers, especially β-cyclodextrin sulfobutyl ether. In addition to simple cyclodextrins, branched cyclodextrins and cyclodextrin polymers may also be used. Other cyclodextrins are described, for example, in *Chemical and Pharmaceutical Bulletin* 28: 1552-1558 (1980); Yakugyo Jiho No. 6452 (28 March 1983); *Angew. Chem. Int. Ed. Engl.* 19: 344-362 (1980); U.S. Patent Nos. 3,459,731 and 4,535,152; European Patent Nos. EP 0 149 197A and EP 0 197 571A; PCT International Patent Publication No. WO90/12035; and UK Patent Publication GB 2,189,245.

References describing cyclodextrins for use in the compositions according to the present invention, and/or which provide a guide for the preparation, purification and analysis of cyclodextrins include the following: *Cyclodextrin Technology* by Jozsef Szejtli, Kluwer Academic Publishers (1988) in the chapter Cyclodextrins in Pharmaceuticals; *Cyclodextrin Chemistry* by M. L. Bender *et al.*, Springer-Verlag, Berlin (1978); *Advances in Carbohydrate Chemistry*, Vol. 12, Ed. By M. L. Wolfrom, Academic Press, New York in the chapter "The Schardinger Dextrins" by Dexter French, pp. 189-260; *Cyclodextrins and their Inclusion Complexes* by J. Szejtli,

Adakemiai Kiado, Budapest, Hungary (1982); I. Tabushi, *Acc. Chem. Research*, 1982, 15, pp. 66-72; W. Sanger, *Angewandte Chemie*, 92, p. 343-361 (1981); A. P. Croft *et al., Tetrahedron*, 39, pp. 1417-1474 (1983); Irie *et al. Pharmaceutical Research*, 5, pp. 713-716 (1988); Pitha *et al., Int. J. Pharm.* 29, 73 (1986); U.S. Patent Nos. 4,659,696 and 4,383,992; German Patent Nos. DE 3,118,218 and DE-3,317,064; and European Patent No. EP 0 094 157A. Patents describing hydroxyalkylated derivative of β- and γ-cyclodextrin include Pitha U.S. Patent Nos. 4,596,795 and 4,727,064, Müller U.S. Patent Nos. 4,764,604 and 4,870,060 and Müller *et al.* U.S. Patent No. 6,407,079.

Amorphous cyclodextrins of particular interest for complexation with cladribine include: hydroxyalkyl, *e.g.* hydroxyethyl or hydroxypropyl, derivatives of β- and γ-cyclodextrin; carboxyalkyl, *e.g.* carboxymethyl or carboxyethyl, derivatives of β- or γ-cyclodextrin; β-cyclodextrin sulfobutyl ether; and randomly methylated β-cyclodextrin. 2-Hydroxypropyl-β-cyclodextrin (HPβCD), 2-hydroxypropyl-γ-cyclodextrin (HPγCD), randomly methylated β-cyclodextrin, β-cyclodextrin sulfobutyl ether, carboxymethyl-β-cyclodextrin (CMβCD) and carboxymethyl-γ-cyclodextrin (CMγCD) are of special interest, especially hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin.

Compositions of an amorphous mixture of amorphous free cladribine and an amorphous, preferably saturated, cladribine-cyclodextrin inclusion complex for use in the present invention can be prepared under conditions favoring complex formation in a liquid environment as described and as exemplified herein. The resultant liquid preparations can be subsequently converted to a dry form suitable for administration as a solid oral or transmucosal dosage form.

One of skill will appreciate that a variety of approaches are available in the field to prepare compositions as described herein. One available

method exemplified herein includes the steps of mixing the cladribine in an aqueous solution of an amorphous cyclodextrin, separating un-dissolved cladribine (*e.g.*, by filtering or centrifugation), and lyophilizing or freeze-drying the saturated solution to form a solid amorphous mixture.

Pharmaceutical compositions according to the invention may optionally include one or more excipients or other pharmaceutically inert components. One of the advantages of the invention, however, is that cladribine drug forms as described herein can be prepared with the minimal amount of excipients necessary for shaping and producing the particular form, such as a tablet or patch. Excipients may be chosen from those that do not interfere with cladribine, with cyclodextrin or with complex formation.

Dosage forms are optionally formulated in a pharmaceutically acceptable vehicle with any of the well-known pharmaceutically acceptable carriers, diluents, binders, lubricants, disintegrants, scavengers, flavoring agents, coloring agents, and excipients (see *Handbook of Pharmaceutical Excipients*, Marcel Dekker Inc., New York and Basel (1998); Lachman *et al.* Eds., *The Theory and Practice of Industrial Pharmacy*, 3rd Ed., (1986); Lieberman *et al.*, Eds. *Pharmaceutical Dosage Forms,* Marcel Dekker Inc., New York and Basel (1989); and *The Handbook of Pharmaceutical Excipients*, 3rd Ed., American Pharmaceutical Association and Pharmaceutical Press, 2000); see also *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, Mack Publishing Co., Easton, PA (1990) and *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams & Wilkins, (1995)). A simple solid oral dosage form consists of the amorphous mixture of amorphous free cladribine and amorphous cladribine-cyclodextrin complex (preferably saturated) as described above, *i.e.* the complex cladribine-cyclodextrin complex, compressed with a small amount (*e.g.* about 1% by weight) of a suitable binder or lubricant such as magnesium stearate.

In certain instances, oral absorption may be further facilitated by the addition of various excipients and additives to increase solubility or to enhance penetration, such as by the modification of the microenvirionment.

The methods and pharmaceutical compositions described herein offer novel therapeutic modalities for the treatment of patients in need of treatment with cladribine. As shown herein, the invention addresses the problems of poor bioavailability traditionally associated with oral cladribine.

The compositions of the invention are particularly suitable as modalities for the treatment of any cladribine-responsive disease. Several disease states responsive to cladribine are well-documented in the literature (see *infra*). For any target disease state, an effective amount of the complex cladribine-cyclodextrin comples, *i.e.* the amorphous mixture of the optimized amorphous saturated cladribine-amorphous cyclodextrin complex with amorphous free cladribine as described above is used (*e.g.*, an amount affective for the treatment of multiple sclerosis, rheumatoid arthritis, or leukemia).

The term "therapeutically effective amount" or "effective amount" is used to denote treatments at dosages effective to achieve the therapeutic result sought. Therapeutically effective dosages described in the literature include those for hairy cell leukemia (0.09 mg/kg/day for 7 days), for multiple sclerosis (from about 0.04 to about 1.0 mg/kg/day (see U.S. Patent No. 5,506,214)); for other diseases, see also U.S. Patent Nos. 5,106,837 (autohemolytic anemia); 5,310,732 (inflammatory bowel disease); 5,401,724 (rheumatoid arthritis); 5,424,296 (malignant astrocytoma); 5,510,336 (histiocytosis); 5,401,724 (chronic myelogenous leukemia); and 6,239,118 (atherosclerosis).

Further, various dosage amounts and dosing regimens have been reported in the literature for use in the treatment of multiple sclerosis; see, for example: Romine et al., *Proceedings of the Association of American*

Physicians, Vol. 111, No. 1, 35-44 (1999); Selby et al., *The Canadian Journal of Neurological Sciences*, 25, 295-299 (1998); Tortorella et al., *Current Opinion in Investigational Drugs*, 2 (12), 1751-1756 (2001); Rice et al., *Neurology,* 54, 1145-1155 (2000); and Karlsson et al., *British Journal of Haematology*, 116, 538-548 (2002); all of which are incorporated by reference herein in their entireties and relied upon.

Moreover, the route of administration for which the therapeutically effective dosages are taught in the literature should be taken into consideration. While the instant compositions optimize the bioavailability of cladribine following oral administration, it will be appreciated that even optimal bioavailability from oral dosage forms is not expected to approach bioavailability obtain after intravenous administration, particularly at early time points. Thus, it is often appropriate to increase a dosage suggested for intravenous administration to arrive at a suitable dosage for incorporation into a solid oral dosage form. At the present time, it is envisioned that, for the treatment of multiple sclerosis, 10 mg of cladribine in the instant complex cladribine-cyclodextrin complex in the instant solid dosage form would be administered once per day for a period of five to seven days in the first month, repeated for another period of five to seven days in the second month, followed by ten months of no treatment. Alternatively the patient would be treated with 10 mg of cladribine in the instant complex cladribine-cyclodextrin complex in the instant dosage form once per day for a period of five to seven days per month for a total of six months, followed by eighteen months of no treatment. For further dosing information, see also U.S. Provisional Patent Application No. _____ [IVAX0021-P-USA/Attorney Docket No. 033935-011], and U.S. Provisional Patent Application No. _____ [IVAX0022-P-USA/Attorney Docket No. 033935-012], both entitled "Cladribine Regimen for Treating Multiple Sclerosis", both filed on March 25, 2004 and incorporated by reference herein in their entireties.

Furthermore, one of skill will appreciate that the therapeutically effective amount of cladribine administered herein may be lowered or increased by fine tuning and/or by administering cladribine according to the invention with another active ingredient. The invention therefore provides a method to tailor the administration/treatment to the particular exigencies specific to a given mammal. Therapeutically effective amounts may be easily determined, for example, empirically by starting at relatively low amounts and by step-wise increments with concurrent evaluation of beneficial effect.

As noted in the preceding paragraph, administration of cladribine in accord with this invention may be accompanied by administration of one or more additional active ingredients for treating the cladribine-responsive condition. The additional active ingredient will be administered by a route of administration and in dosing amounts and frequencies appropriate for each additional active ingredient and the condition being treated. For example, in the treatment of multiple sclerosis, other useful drugs include interferon beta (Rebif®, Betaseron®/Betaferon®, Avonex®), identical to the naturally occurring protein found in the human body; glatiramer acetate (Copaxone®), a random chain (polymer) of the amino acids glutamic acid, lysine, alanine and tyrosine; natalizumab (Antegren®), a monoclonal antibody; alemtuzumab (Campath-1H®), a humanized anti-CD52 monoclonal antibody; 4-aminopyridine (also known as 4-AP and Fampridine), a drug that blocks the potassium channels in neurons; and amantadine, an anti-viral agent which improves muscle control and reduces muscle stiffness and is used to alleviate the symptoms of fatigue in multiple sclerosis, a purpose for which pemoline (Cylert®) and L-Carnitine (a herbal product) may also be useful. In the treatment of hairy cell leukemia, additional active ingredients may include interferon alpha, pentostatin, fludarabine, rituximab (an anti-CD 20 monoclonal antibody) and the anti-CD22 recombinant immunotoxin BL 22; other additional active ingredients may be appropriate in other types of

leukemias. In the treatment of rheumatoid arthritis, there are many other active ingredients which may be selected. These include NSAIDS (non-steroidal anti-inflammatory drugs), which are of three types: salicylates such as aspirin, traditional NSAIDS such as ibuprofen and indomethacin, and COX-2 inhibitors such as celecoxib (Celebrex®), rofecoxib (Vioxx®), meloxicam (Mobic®), valdecoxib (Bextra®), lumiracoxib (Prexige®) and etoricoxib (Arcoxia®). Other drugs useful in treating rheumatoid arthritis which may be used in conjunction with the present invention include DMARDS, glucocorticoids, biological response modifiers and non-NSAID analgesics. DMARDS are disease-modifying anti-rheumatic drugs which include methotrexate, plaquenil, leflunomide (Arava®), sulfasalazine, gold, penicillamide, cyclosporine, methyl cyclophosamide and azathioprine. Glucocorticoids include dexamethasone, prednisolone, triamcinolone and many others. Biological response modifiers (which restore the disease-fighting ability of the immune system), include etanercept (Enrel®), a tumor-necrosis factor inhibitor, infliximab (Remicade®), which is also an anti-TNF drug, anakinra (Kineret®), a selective IL-1 blocker, and Humira®, a human monoclonal antibody which is another anti-TNF drug. The non-NSAID analgesics include acetaminophen as well as narcotic analgesics such as hydrocodone, oxycodone and propoxyphene. Generally speaking, those drugs which work by a mechanism different from that of cladribine are particularly useful for concomitant therapy with the cladribine composition described herein. Those drugs which are effective by the oral route of administration and which are compatible with the instant cladribine complexes in a single dosage form may be incorporated into the instant dosage forms; otherwise, they should of course be separately administered in amounts, frequencies and via administration routes suitable to them.

As used herein, "treating" means reducing, preventing, hindering the development of, controlling, alleviating and/or reversing the symptoms in the individual to which a compound of the invention has been administered, as

compared to the symptoms of an individual not being treated according to the invention. A practitioner will appreciate that the complexes, compositions, dosage forms and methods described herein are to be used in concomitance with continuous clinical evaluations by a skilled practitioner (physician or veterinarian) to determine subsequent therapy. Such evaluation will aid and inform in evaluating whether to increase, reduce or continue a particular treatment dose, and/or to alter the mode of administration.

The methods of the present invention are intended for use with any subject/patient that may experience the benefits of the methods of the invention. Thus, in accordance with the invention, the terms "subjects" as well as "patients" include humans as well as non-human subjects, particularly domesticated animals.

Any suitable materials and/or methods known to those of skill can be utilized in carrying out the present invention. However, preferred materials and methods are described. Materials, reagents and the like to which reference are made in the following description and examples are obtainable from commercial sources, unless otherwise noted.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein.

EXAMPLES

EXAMPLE 1

PHASE SOLUBILITY STUDY

A phase solubility study can be carried out as follows. Excess cladribine is added to cyclodextrin solutions of various concentrations of

hydroxypropyl-β-cyclodextrin (HPβCD) and allowed to complex as described in EXAMPLE 2 below. The excess, undissolved cladribine is removed by filtration. The amount of cladribine in the complexation solution is measured to obtain a data point. This process is repeated with different known concentrations of HPβCD until several data points are obtained. These data points are then plotted graphically, each data point representing the amount of cladribine that can be dissolved in water with a specific concentration of cyclodextrin. Points on the line generated by the data points represent ratios for the product. One of skill in the art will realize the same results will be generated if excess cyclodextrin is added to cladribine solutions of known concentration.

The molar concentrations of cladribine to cyclodextrin obtained are plotted and presented graphically. A representative phase solubility diagram is shown in the Figure. The plotted lines for cladribine-HPβCD represent cladribine solubilization for the conditions tested, that is, the ratio of the concentration of cladribine to the concentration of cyclodextrin. The area above each of the plotted lines represents conditions where excess insoluble cladribine is present. The area below each of the plotted lines represents the conditions where cyclodextrin is in excess.

The plot for cladribine-HPβCD shown in the Figure is approximately linear; this is indicative of a 1:1 complex, in which one molecule of the drug is complexed with one molecule of cyclodextrin. The Figure also shows that additional cyclodextrin is needed to maintain the cladribine in the complex. For example, about 0.14 mole of HPβCD is needed to maintain about 0.049 mole of cladribine dissolved under the selected conditions, which will ultimately provide the amorphous mixture of the amorphous, preferably saturated, cladribine-HPβCD inclusion complex and amorphous free cladribine (as a non-inclusion complex). Under the conditions of EXAMPLE 2 below, a significant portion of the cladribine in the product can be expected to be not in the inclusion complex but rather in amorphous form loosely held

in intimate admixture therewith by hydrogen bonding as a non-inclusion complex.

EXAMPLE 2

PREPARATION OF CLADRIBINE-CYCLODEXTRIN COMPLEX FOR HUMAN TRIALS

Cladribine is complexed with HPβCD by the following method.

In 825 mL of distilled water, 172.5 g of hydroxypropyl-β-cyclodextrin are dissolved (forming an approximately 17% solution), then cladribine is added and the mixture is stirred at about 45 to about 50°C for about nine hours. Stirring is continued for an additional 6 to 9 hours at room temperature. Any undissolved cladribine is removed by filtration and the solution is cooled to room temperature. To form the amorphous mixture of amorphous cladribine-cyclodextrin complex and amorphous free cladribine, the aqueous cladribine-cyclodextrin solution is dried by lyophilization prior to incorporation into solid oral tablets. The lyophilization procedure comprises a freezing stage of rapidly bringing the complexation solution to about -40°C to about -80°C (e.g., about -45°C) for approximately 2 to 4 hours (preferably about 3 to 4 hours), followed by a primary drying stage at about -25°C for approximately 80-90 hours, typically under low pressure, and a second drying stage at about 30°C for about 15-20 hours.

Product made by the foregoing general procedure can be analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based mobile phase, with UV detection at 264 nm) to find the weight ratio of cladribine to cyclodextrin in the final product. Final product preparations can be further characterized by methods known in the art, including, for example by inspecting appearance, ascertaining the overall impurity content by HPLC, ascertaining the water content using a Karl Fischer titrator, determining the dissolution profile by a standard method, for example using USP<711>Apparatus II equipment and UV detection at 264 nm, inspecting

the content uniformity and performing quantitative assay by HPLC analysis of the active ingredient.

Two batches of cladribine/cyclodextrin product, FD04 and FD05, were made by the foregoing general procedure as follows:

Purified water (825 mL) was pre-heated at 48°C (target range 45°C to 50°C) in a 1-liter glass vessel by immersion in a water bath. The heated water was stirred to achieve a controlled central vortex. 2-hydroxypropyl-β-cyclodextrin (172.50 g) was weighed and slowly added to the heated water over a period of 40 minutes. The resulting solution was stirred for a further 10 minutes to ensure complete dissolution of the cyclodextrin. Cladribine (12.00 g for FD04 and 18.75 g for FD05) was weighed and added to the stirred cyclodextrin solution, which turned cloudy before becoming clear. The resulting clear solution was maintained at 48°C and continually stirred for 9 hours. Stirring continued for a further 7 hours while the solution cooled to room temperature.

Use of a larger amount of cladribine in the preparation of FD05 was part of an attempt to optimize the procedure; however, it was found that the initial amount of cladribine in that case was too great and precipitation was observed at the end of the cooling step for batch FD05. The solution was filtered to remove the precipitate. Analysis of the resultant product revealed (assay value = 87.2%) that 16.35 g of cladribine had been incorporated into the cyclodextrin complex in the case of FD05. No filtration was required for batch FD04, indicating that the amounts used in the preparation of FD04 were more appropriate and that the FD05 procedure could be optimized by beginning with a smaller amount of cladribine (16.35 g rather than 18.75 g), thus avoiding the filtration step.

After cooling to room temperature and, in the case of FD05, filtering, the solutions were filled into 100 mL lyophilization vials (20 mL solutions per vial), the filled vials were partially stoppered and lyophilized. The

lyophilization included freezing at -45°C for about 200 minutes, a primary drying phase at -25°C under a pressure of 100 mTorr for about 5,200 minutes and a secondary drying phase at 30°C for about 1,080 minutes as set forth below:

TABLE I

Step	Process	Temperature	Pressure (mTorr)	Time (min)
1	Load	4°C		
2	Load Hold	4°C	n/a	120
3	Ramp	-45°C	n/a	120
4	Freezing	-45°C	n/a	200
5	Ramp	-25°C	100	120
6	Primary drying	-25°C	100	5200
7	Ramp	30°C	50	240
8	Secondary drying	30°C	50	1080
9	Finish	30°C	Vials closed under vacuum	

The FD04 and FD05 batches of cladribine/cyclodextrin product made by the foregoing procedure were analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based mobile phase with UV detection at 264 nm) and empirically found to have the following characteristics:

TABLE II

Lot No.	Cladribine: HPβCD w/w	Cladribine: HPβCD Weight Ratio
FD04	12.00g:172.50g	1:14.38
FD05	16.35g:172.50g	1:10.55

The products were analyzed by DSC thermograms and X-ray diffraction methods to determine any free crystalline cladribine in the lyophilized material. Importantly, the samples exhibited no transitions in the region of 210°C to 230°C, which is associated with the melting of crystalline cladribine. In both cases, no significant thermal activity was recorded in the range of 210°C to 230°C, suggesting that the complexes obtained at the end of the lyophilization do not have any significant amount of free crystalline cladribine, considering the sensitivity of the analytical method (up to 3% w/w). This conclusion was supported by the absence of peaks for crystalline cladribine from X-ray diffraction traces for both complexes FD04 and FD05.

The products are amorphous mixtures of amorphous cladribine-HPβCD inclusion complex and amorphous free cladribine hydrogen-bonded to the cyclodextrin as a non-inclusion complex. The cladribine:HPβCD weight ratios obtained were about 1:14 and 1:11.

Generally speaking, amorphous mixtures within the scope of the present invention have cladribine:HPβCD weight ratios of from about 1:10 to 1:16.

EXAMPLE 3

PREPARATION OF ORAL TABLETS

Tablets were manufactured using batches of amorphous mixtures FD04 and FD05 described in EXAMPLE 2 for use in a clinical study.

Batch N0120 was manufactured using cladribine-2-HPβCD complex mixture DF05 to a batch size of 3,000 tablets and batch N0126 was manufactured using cladribine-HPβCD complex mixture FD04 to a batch size of 800 tablets. The master formulations for the two batches are shown in TABLE III. Batch N0120 represented 3.0 g tablets and Batch N0126 represented 10 mg tablets for clinical study.

TABLE III

Constituent	Lot Number	mg/tablet 3.0 mg Batch N0120	mg/tablet 10.0 mg Batch N0126
Cladribine-HPβCD complex mix	FD05	30.60*	
Cladribine-HPβCD complex mix	FD04		153.75**
Sorbitol powder NF	1007403	68.4	44.25
Magnesium stearate NF	1006280	1.00	2.00
Total		100.00	200.00

*Equivalent to 3.0 mg cladribine per tablet.
**Equivalent to 10.0 mg cladribine per tablet.

The following table sets forth the method of manufacture of the Batch N0120 and N0126 tablets.

TABLE IV

1.	Pre-mix the magnesium stearate with an approximately equal quantity of sorbitol power.
2.	Pass the cladribine-HPβCD complex and the remainder of the sorbitol powder into a one-liter glass jar via a 40-mesh screen.
3.	Blend the contents for 10 minutes at 12 rpm.
4.	Pass the magnesium stearate/sorbitol powder pre-mix into the glass jar via the 40-mesh screen.
5.	Blend the final mixture for 5 minutes at 12 rpm.
6.	Compress into 3.0 mg and 10.0 mg tablets at a target compression weight of 100 mg and 200 mg, respectively.

Both the Batch N0120 3.0 mg tablets and the Batch N0126 10.0 mg tablets were round, with one side flat-beveled edged and the other side shallow convex. The Batch N0120 3.0 mg tablets had an average weight of 100 mg, a thickness of 2.7 mm, a friability of 0.2%, a hardness of 4 Kp and a disintegration time of 3 minutes. The Batch N0126 10.0 mg tablets had an average weight of 198 mg, a thickness of 4.2 mm, a friability of 1%, a hardness of 2.8 Kp and a disintegration time of 5 minutes 42 seconds.

The Batch N0120 3.0 mg and N0126 10.0 mg tablets were used in the clinical study summarized in EXAMPLE 5 below.

EXAMPLE 4

CLINICAL STUDY: RELATIVE BIOAVAILABILITY

The objective of this study was to assess the relative bioavailability of three oral cladribine formulations: (1) a cyclodextrin-based formulation according to the instant invention (Tablet 1: complex FD05, i.e. Batch No. N0120 tablets described above); (2) a mucoadhesive formulation (Tablet 2: containing 3.0 mg cladribine, 10 mg of Carbopol 71G NF, 22.2 mg of dicalcium phosphate, 64.3 mg of lactose and 0.5 mg of magnesium stearate, Batch No. N0121); and (3) a hard-gel capsule (Capsule containing 3.0 mg cladribine, 5.0 mg Carbopol 974P, 91.3 mg Avicel PH101, 100.0 mg Avicel PH102, 0.2 mg colloidal silicon dioxide and 0.5 mg magnesium stearate, Batch No. RD03030) in comparison with one fixed subcutaneous clardribine administration (reference formulation) in patients with MS (multiple sclerosis).

This study was a 2 center, open-label, randomized, 4-way crossover single dose study using twelve patients with MS. Patients received randomly three different fixed oral doses (3.0 mg) and a fixed subcutaneous dose of 3.0 mg. The four treatment days were separated by a drug-free interval of at

least 5 days. In each treatment period, blood samples were collected over a 24-hour period for evaluation of plasma cladribine.

The plasma concentration of cladribine was measured by a HPLC/MS/MS method. Using this method, the relationship between concentration versus peak area ratio was found to be linear within the range of 100 pg/ml to 50,000 pg/ml for cladribine. The limit of quantification was 100 pg/ml. Analysis of samples was carried out in 16 runs. No calibrator had to be excluded from fitting of the calibration curve and accuracy of each quality control sample met the GLP requirements.

576 clinical plasma samples were analyzed and concentration values of cladribine were determined. The results were compiled and are summarized in the tables below (Tables V and VI). In these tables, the following definitions are applicable: T_{max} is the time to reach maximum concentration in the plasma; $T_{½}$ is the half-life of cladribine in the plasma; C_{MAX} is the maximum concentration of cladribine in the plasma; AUC_{inf} is the area under the curve for the measured data from zero extrapolated to infinity; AUC_t is the area under the curve for the measured data (from zero to the last time point); Geom Mean is the geometric mean; CV is the coefficient of variation (relative standard deviation); LL is the lower limit; UL is the upper limit.

TABLE V
Summary Statistics for Pharmacokinetic Parameters for Cladribine Study Obtained via Non-Compartmental Analyses. (n=12).

Pharmacokinetic Parameter	3.0 mg subcutaneous			3mg Tablet 1			3mg Tablet 2			3 mg Capsule		
	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)
T_{max}(hr)	N/A	.313 ±.113	36.2	N/A	.521 ±.167	32.1	N/A	1.25 ±.839	67.1	N/A	2.25 ±.622	27.7
$T_{½}$(hr)	N/A	6.69 ±2.01	30.1	N/A	7.55 ±2.50	33.1	N/A	6.73 ±2.82	41.9	N/A	6.27 ±2.31	36.9
C_{max}(pg/ml)	23186	N/A	40.1	6597	N/A	24.7	5041	N/A	52.6	3818	N/A	36.8
AUC_{inf} (hr·pg/ml)	57254	N/A	44.4	24936	N/A	28.8	21676	N/A	42.7	22604	N/A	39.5
AUC_t (hr·pg/ml)	54725	N/A	43.8	23182	N/A	28.0	20063	N/A	42.1	20951	N/A	42.0

**CV=SD/mean for T_{max} and $T_{½}$ and CV% geometric mean for C_{max}, AUC_{inf} and AUC_t.

TABLE VI

Ratios of Oral to Subcutaneous Pharmacokinetic Parameters and Corresponding Two-Sided 90% Confidence Intervals for Cladribine Study (n=12).

Pharmacokinetic Parameter	3 mg Tablet 1		3mg Tablet 2		3mg Capsule	
	Ratio*	LL, UL	Ratio*	LL, UL	Ratio*	LL, UL
AUC_{inf}	43.1	35.7, 52.1	38.4	31.8, 46.4	38.9	32.1, 47.0
AUC_t	41.9	34.6, 50.8	37.2	30.7, 45.0	37.6	31.0, 45.5

*Ratios (dose normalized) and Corresponding 95% LL obtained via inverse transformation of log-transformed data.

EXAMPLE 5

CLINICAL STUDY: DOSE RESPONSE AND ABSOLUTE BIOAVAILABILITY

The objective of this study was to assess the systemic availability of cladribine after oral administration in two different fixed oral doses, in comparison with one fixed intravenous administration (reference formulation) in patients with MS (multiple sclerosis), and to evaluate the safety and tolerability of cladribine in this population.

This study was a 3 center, open-label, randomized, 3-way crossover single dose study using twenty-six patients with MS. Patients received randomly two different fixed oral doses (3.0 mg and 10.0 mg) and a fixed intravenous dose of 3.0 mg (administered as a 1 hour infusion). The three treatment days were separated by a drug-free interval of at least 5 days. In

each treatment period blood samples were collected over a 24-hour period for evaluation of plasma cladribine.

The plasma concentrations of cladribine were measured by a HPLC/MS/MS method. Using this method the relationship between concentrations versus peak area ratios was found to be linear within the range of 100 pg/ml to 50,000 pg/ml for cladribine. The limit of quantification was 100 pg/ml. Analysis of samples was carried out in 16 runs. Except the first run (which had to be rejected because of equipment failure), all other runs could be accepted. No calibrator had to be excluded from fitting of the calibration curve and accuracy of each quality control sample met the GLP requirements.

858 clinical plasma samples were analyzed and concentration values of cladribine were determined. The results were compiled and are summarized in the tables below [TABLES VII through X]. In these tables, the following definitions are applicable: T_{max} is the time to reach maximum concentration in the plasma; $T_{1/2}$ is the half-life of cladribine in the plasma; C_{max} is the maximum concentration of cladribine in the plasma; AUC_{inf} is the area under the curve for the measured data from zero extrapolated to infinity; AUC_t is the area under the curve for the measured data (from zero to the last time point); Geom Mean is the geometric mean; CV is the coefficient of variation (relative standard deviation); LL is the lower limit; UL is the upper limit; σ^2 is the mean variance; σ_B^2 is the mean variance between subjects; σ_W^2 is the mean variance within subjects; CV_T is the total coefficient of variation; and CV_W is the coefficient of variation within subjects.

TABLE VII

Summary Statistics for Pharmacokinetic Parameters for Cladribine Study Obtained via Non-Compartmental Analysis (n=26)

Pharmaco-kinetic Parameter	3.0 mg IV infusion			Oral Administration					
				3.0 mg			10.0 mg		
	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)
T_{max}(hr)	N/A	.817 ±.397	48.6	N/A	.548 ±.300	54.8	N/A	.558 ±.204	36.5
$T_{½}$(hr)	N/A	6.50 ±1.27	19.5	N/A	5.85 ±1.18	20.2	N/A	5.60 ±0.75	13.3
C_{max}(pg/ml)	21425	N/A	27.6	5608	N/A	49.5	21242	N/A	50.5
AUC_{inf} (hr·pg/ml)	58528	N/A	24.0	20159	N/A	35.0	76690	N/A	30.3
AUC_t (hr·pg/ml)	56396	N/A	24.0	19166	N/A	36.9	74532	N/A	30.3

**CV=SD/mean for T_{max} and $T_{½}$ and CV% geometric mean for C_{max}, AUC_{inf} and AUC_t.

TABLE VIII

Ratios of Oral to I.V. Pharmacokinetic Parameters and Corresponding Lower Limit (LL) for the one-sided 95% Confidence Interval for Cladribine Study (n=26)

Pharmacokinetic Parameter	Oral Administration			
	3.0 mg		10.0 mg	
	Ratio*	LL	Ratio*	LL
AUC_{inf}	34.5	31.7	39.1	35.9
AUC_t	34.0	31.2	39.4	36.1

*Ratios (dose normalized) and Corresponding 95% LL obtained via inverse transformation of log-transformed data.

TABLE IX

Ratios and Corresponding two-sided 90% Confidence Intervals for Cladribine Study (n=26)

Pharmacokinetic Parameter	10.0 mg/3.0 mg		
	Ratio*	LL	UL
C_{max}	112.6	95.1	133.3
AUC_{inf}	113.3	104.2	123.3
AUC_t	115.8	106.1	126.5

*Ratios (dose normalized) and Corresponding 90% CI obtained via inverse transformation of log-transformed data.

TABLE X

Variance components for Cladribine Study (n=26)

Source of variation	C_{max}	AUC_{inf}	AUC_t
Between (σ_B^2)	.0380	.0487	.0492
With (σ_W^2)	.1315	.0330	.0357
TOTAL ($\sigma_B^2 + \sigma_W^2$)	.1695	.0816	.0849
CV_T(%)	43.0	29.2	29.8
CV_W(%)	37.5	18.3	19.1

Where PK parameters are dose-adjusted and $CV = \sqrt{\exp(\sigma^2) - 1}$

The foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents thereof may be resorted to, falling within the scope of the invention claimed.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

2. The pharmaceutical composition according to Claim 1, wherein the complex is saturated with cladribine.

3. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

4. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

5. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

6. The composition according to any one of Claims 1 to 3, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

7. The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

8. The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

9. The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

10. The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

11. The composition according to any one of Claims 1 to 10, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

12. The composition according to any one of Claims 1 to 11, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

13. A method for enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

14. The method according to Claim 13, wherein the complex is saturated with cladribine.

15. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

16. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

17. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

18. The method according to any one of Claims 13 to 15, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

19. The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

20. The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

21. The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

22. The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

23. The method according to any one of Claims 13 to 22, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin

corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

24. The method according to any one of Claims 13 to 23, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

25. A method for the treatment of symptoms of a cladribine-responsive condition in a subject suffering from said symptoms comprising orally administering to said subject a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

26. The method according to Claim 25, wherein the complex is saturated with cladribine.

27. The method according to Claim 25 or 26, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

28. The method according to Claim 27, wherein the cladribine-responsive condition is multiple sclerosis.

29. The method according to Claim 25, 26, 27 or 28, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin,

hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

30. The method according to any one of Claims 25 to 29, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

31. The method according to any one of Claims 25 to 30, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

32. The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

33. The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

34. The method according to Claim 25, 26, 27 or 28, wherein the amorphous cyclodextrin is hydropropyl-γ-cyclodextrin.

35. The method according to any one of Claims 25 to 34, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

36. Use of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, in the formulation of a solid oral dosage form, for administration in the treatment of symptoms of a cladribine-responsive condition.

37. Use according to Claim 36, wherein the complex is saturated with cladribine.

38. Use according to Claim 36 or 37, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

39. Use according to Claim 38, wherein the cladribine-responsive condition is multiple sclerosis.

40. Use according to Claim 36, 37, 38 or 39, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

41. Use according to any one of Claims 36 to 40, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

42. Use according to any one of Claims 36 to 41, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

43. Use according to Claim 42, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

44. Use according to Claim 42, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

45. Use according to any one of Claims 36 to 41, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

46. Use according to any one of Claims 36 to 45, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

47. Use of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, in the formulation of a solid oral dosage form, for enhancing the oral bioavailability of cladribine.

48. Use according to Claim 47, wherein the complex is saturated with cladribine.

49. Use according to Claim 47 or 48, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

50. Use according to any one of Claims 47 to 49, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

51. Use according to any one of Claims 47 to 50, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

52. Use according to Claim 51, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

53. Use according to Claim 51, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

54. Use according to any one of Claims 47 to 50, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

55. Use according to any one of Claims 47 to 54, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

56. A complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex.

57. The complex according to Claim 56, saturated with cladribine.

58. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

59. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

60. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

61. The complex according to any one of Claims 56 to 58, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

62. The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

63. The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

64. The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

65. The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

66. The complex according to any one of Claims 56 to 65, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

67. A process for the preparation of a complex cladribine-cyclodextrin complex which comprises the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature; and

(iii) lyophilizing the cooled solution to afford an amorphous product.

68. A process according to Claim 67, further comprising a filtration step following step (ii).

69. A process according to Claim 67 or 68, wherein step (i) is performed at a temperature of from about 45 to about 60°C.

70. A process according to any one of Claims 67 to 69, wherein step (i) is performed at a temperature of from about 45 to about 50°C.

71. A process according to Claim 69 or 70, wherein step (i) is performed with stirring.

72. A process according to Claim 71, wherein step (i) is performed for a period of from about 6 to about 9 hours.

73. A process according to any one of Claims 67 to 72, wherein step (ii) is performed for a period of from about 6 to about 9 hours.

74. A process according to any one of Claims 67 to 73, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80° C, and held at said temperature for a period of from about 2 to about 4 hours.

75. A process according to Claim 74, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

76. A process according to any one of Claims 67 to 75, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

77. A process according to any one of Claims 67 to 75, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

78. A process according to Claim 76 or 77, wherein 825 parts by volume of water are introduced in step (i).

79. A process according to any one of Claims 67 to 78, wherein the lyophilization step (iii) comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

80. A process according to Claim 79, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

81. A process according to Claim 79 or 80, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

82. A pharmaceutical composition obtainable by a process comprising the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature;

(iii) lyophilizing the cooled solution to afford an amorphous product; and

(iv) formulating the amorphous product into a solid oral dosage form.

83. A pharmaceutical composition according to Claim 82, wherein the process further comprises a filtration step following step (i) or (ii).

84. A pharmaceutical composition according to Claim 82 or 83, wherein step (i) of the process is performed at a temperature of from about 45 to about 60°C.

85. A pharmaceutical composition according to any one of Claims 82 to 84, wherein step (i) of the process is performed at a temperature of from about 45 to about 50°C.

86. A pharmaceutical composition according to Claim 84 or 85, wherein step (i) of the process is performed with stirring.

87. A pharmaceutical composition according to Claim 86, wherein step (i) of the process is performed for a period of from about 6 to about 9 hours.

88. A pharmaceutical composition according to any one of Claims 82 to 87, wherein step (ii) of the process is performed for a period of from about 6 to about 9 hours.

89. A pharmaceutical composition according to any one of Claims 82 to 88, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80°C, and held at said temperature for a period of from about 2 to about 4 hours.

90. A pharmaceutical composition according to Claim 89, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

91. A pharmaceutical composition according to any one of Claims 82 to 90, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

92. A pharmaceutical composition according to any one of Claims 82 to 90, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

93. A pharmaceutical composition according to Claim 91 or 92, wherein 825 parts by volume of water are introduced in step (i) of the process.

94. A pharmaceutical composition according to any one of Claims 82 to 93, wherein the lyophilization step (iii) of the process comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

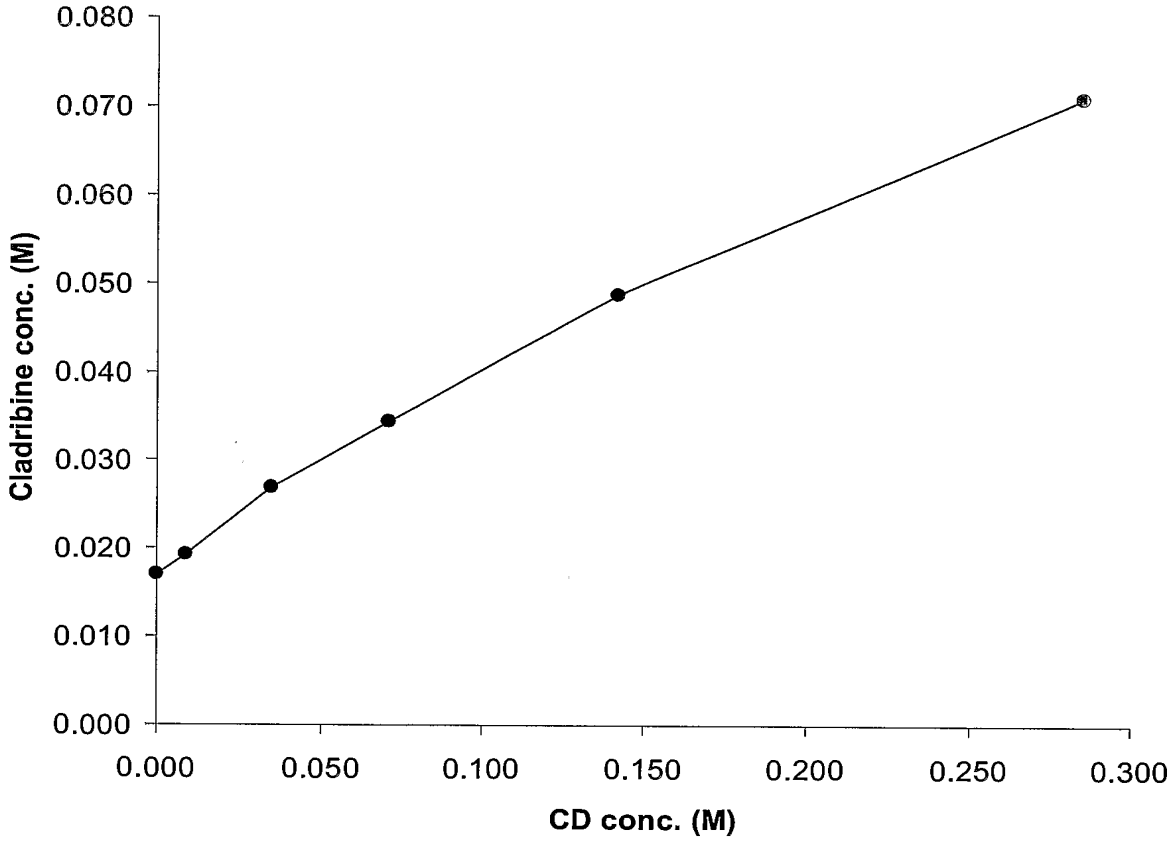
(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

95. A pharmaceutical composition according to Claim 94, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

96. A pharmaceutical composition according to Claim 94 or 95, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

97. A pharmaceutical composition according to any one of Claims 82 to 96, wherein the formulation step (iv) of the process comprises blending the complex with magnesium stearate and compressing into tablets.

98. A pharmaceutical composition according to Claim 97, wherein magnesium stearate is pre-mixed with sorbitol powder before blending with the complex.


0.080
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Cladribine conc. (M)
0.000
0.050
0.100
0.150
0.200
0.250
0.300
CD conc. (M)

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	GIAMPIERO DE LUCA
Filer:	Frank Christopher Eisenschenk/Sherry Loke
Attorney Docket Number:	SER-125

Filed as Large Entity

U.S. National Stage under 35 USC 371 Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
National Stage Fee	1631	1	300	300
Natl Stage Search Fee - Report provided	1642	1	400	400
National Stage Exam - all other cases	1633	1	200	200
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**900**

Electronic Acknowledgement Receipt

EFS ID:	1881928
Application Number:	11722018
International Application Number:	PCT/EP05/56954
Confirmation Number:	5532
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	GIAMPIERO DE LUCA
Customer Number:	23557
Filer:	Frank Christopher Eisenschenk/Sherry Loke
Filer Authorized By:	Frank Christopher Eisenschenk
Attorney Docket Number:	SER-125
Receipt Date:	18-JUN-2007
Filing Date:	
Time Stamp:	14:43:50
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

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Payment was successfully received in RAM	$900
RAM confirmation Number	6690
Deposit Account	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)	Multi Part /.zip	Pages (if appl.)

1		PreAmd.pdf	302711	yes	8

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Preliminary Amendment	1	1
Specification	2	2
Claims	3	6
Applicant Arguments/Remarks Made in an Amendment	7	7
Abstract	8	8

Warnings:					
Information:					
2	Application Data Sheet	ADS.pdf	95541	no	3
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
3	Oath or Declaration filed	executed-Dec.pdf	77284	no	2
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Filed	IDS.pdf	575393	no	4
Warnings:					
Information:					
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5	NPL Documents	BEUTLER-1.pdf	2258023	no	6
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Information:					
6	NPL Documents	BEUTLER-2.pdf	733855	no	8
Warnings:					

Information:					
7	NPL Documents	BEUTLER-3.pdf	636437	no	5
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8	NPL Documents	ELLISON.pdf	298199	no	2
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9	Foreign Reference	EP00626853.pdf	65243	no	14
Warnings:					
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10	NPL Documents	GRIEB.pdf	821536	no	5
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11	NPL Documents	KAZIMIERCZUK.pdf	543725	no	4
Warnings:					
Information:					
12	NPL Documents	KURTZKE.pdf	888621	no	9
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18	NPL Documents	MCDONALD.pdf	612160	no	7
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19	NPL Documents	MILLER.pdf	141122	no	1
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27	NPL Documents	STELMASIAK.pdf	593050	no	5
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28	Foreign Reference	WO04087101.pdf	2796486	no	56
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Information:					
29	Fee Worksheet (PTO-06)	fee-info.pdf	8433	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	24474778		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on August 16, 2007.

Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

Patent Application
Docket No. SER-125
Serial No. 11/722,018

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Giampiero De Luca
Serial No.	:	11/722,018
Filed	:	June 18, 2007
Conf. No.	:	5532
For	:	Cladribine Regimen for Treating Multiple Sclerosis

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUBMISSION OF POWER OF ATTORNEY AND CORRESPONDENCE ADDRESS INDICATION FORM

Sir:

Transmitted herewith for filing in connection with the above-identified patent application are Power of Attorney and Correspondence Address Indication Forms executed by the inventor.

Respectfully submitted,

Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D.
Patent Attorney
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Phone No.: 352-375-8100
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Address: P.O. Box 142950
Gainesville, FL 32614-2950

FCE/jps
Attachments: Power of Attorney forms

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PTO/SB/81 (04-05)
Approved for use through 11/30/2005. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

POWER OF ATTORNEY and CORRESPONDENCE ADDRESS INDICATION FORM

Application Number	11/722,018
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Title	Cladribine Regimen for Treating ...
Art Unit	
Examiner Name	
Attorney Docket Number	SER-125

I hereby revoke all previous powers of attorney given in the above-identified application.

I hereby appoint:

☑ Practitioners associated with the Customer Number: 23557

OR

☐ Practitioner(s) named below:

Name	Registration Number

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please recognize or change the correspondence address for the above-identified application to:

☑ The address associated with the above-mentioned Customer Number:

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Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☑ Applicant/Inventor.

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

SIGNATURE of Applicant or Assignee of Record

Signature		Date	12/07/2007
Name	GIAMPIERO DE LUCA	Telephone	
Title and Company			

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

Electronic Acknowledgement Receipt

EFS ID:	2092294
Application Number:	11722018
International Application Number:	
Confirmation Number:	5532
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	GIAMPIERO DE LUCA
Customer Number:	23557
Filer:	Frank Christopher Eisenschenk/Sherry Loke
Filer Authorized By:	Frank Christopher Eisenschenk
Attorney Docket Number:	SER-125
Receipt Date:	16-AUG-2007
Filing Date:	
Time Stamp:	16:24:21
Application Type:	U.S. National Stage under 35 USC 371

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Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes) /Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	TransLtr-POA.pdf	134710 1eafeb41f806bd7797f1e3f2666b9ffa4ebd0b20	no	2

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New Applications Under 35 U.S.C. 111

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National Stage of an International Application under 35 U.S.C. 371

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New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

11/732018

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/EP2005/056954

International filing date: 20 December 2005 (20.12.2005)

Document type: Certified copy of priority document

Document details: Country/Office: EP
Number: 04106909.7
Filing date: 22 December 2004 (22.12.2004)

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Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

Europäisches Patentamt	European Patent Office	Office européen des brevets

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.	The attached documents are exact copies of the European patent application described on the following page, as originally filed.	Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr.	Patent application No.	Demande de brevet n°
	04106909.7	

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
Application no.: 04106909.7
Demande no:

Anmeldetag:
Date of filing: 22.12.04
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Applied Research Systems ARS Holding N.V.
Pietermaai 15
Curacao
ANTILLES NEERLANDAISES

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Cladribine regimen for treating Multiple Sclerosis

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K31/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LT LU
MC NL PL PT RO SE SI SK TR LI

Cladribine regimen for treating Multiple Sclerosis

Field of the Invention

The present invention relates to the use of multiple doses of Cladribine for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis.

Background of the Invention

Multiple sclerosis (MS) is the most known chronic inflammatory demyelinating disease of the central nervous system in humans. The onset of the disease typically occurs during ages 20 to 40. Women are affected approximately twice as often as men.

Over time, MS may result in the accumulation of various neurological disabilities. Clinical disability in MS is presumed to be a result of repeated inflammatory injury with subsequent loss of myelin and axons, leading to tissue atrophy.

MS is manifested in physical symptoms (relapses and disability progression), Central Nervous System (CNS) inflammation, brain atrophy and cognitive impairment. Presenting symptoms include focal sensory deficits, focal weakness, visual problems, imbalance and fatigue. Sexual impairment and sphincter dysfunction may occur. Approximately half of the patients with MS may experience cognitive impairment or depression.

MS is now considered to be a multi-phasic disease and periods of clinical quiescence (remissions) occur between exacerbations. Remissions vary in length and may last several years but are infrequently permanent.

Four courses of the disease are individualized: relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR) multiple sclerosis.

More than 80% of patients with MS will initially display a RR course with clinical exacerbation of neurological symptoms, followed by a recovery that may or may not be complete (*Lublin and Reingold, Neurology, 1996, 46:907-911*).

During RRMS, accumulation of disability results from incomplete recovery from relapses. Approximately, half of the patients with RRMS switch to a progressive course, called SPMS, 10 years after the diseased onset. During the SP phase, worsening of disability results from the accumulation of residual symptoms after exarcerbation but also from insidious progression between exacerbations (*Lublin and Reingold above).* 10% of MS patients have PPMS which is characterized by insidious progression of the symptoms from the disease onset. Less than 5 % of patients have PRMS and are often considered to have the same prognosis as PPMS. It is suggested that distinct pathogenic mechanisms may be involved in different patient sub-groups and have wide-ranging implications for disease classification (*Lassmann et al., 2001, Trends Mol. Med., 7, 115-121; Lucchinetti et al., Curr. Opin. Neurol., 2001, 14, 259-269*).

MS onset is defined by the occurrence of the first neurological symptoms of CNS dysfunction. Advances in cerebrospinal fluid (CSF) analysis and magnetic resonance imaging (MRI) have simplified the diagnostic process and facilitated early diagnostic (*Noseworthy et al., The New England Journal of Medicine, 2000, 343, 13, 938-952*). The International Panel on the Diagnosis of MS issued revised criteria facilitating the diagnosis of MS and including MRI together with clinical and para-clinical diagnostic methods (*Mc Donald et al., 2001, Ann. Neurol., 50:121-127*).

Current medications for MS which are disease modifying treatments, i.e. modifying the course of MS, modulate or suppress the immune system. There are four FDA approved immunomodulating agents for RRMS: three beta interferons (Betaseron®, Berlex; Avonex®, Biogen; Rebif®, Serono) and Glatimarer Acetate (Copaxone®, Amgen). There is also one FDA approved immunosuppressing drug for worsening MS, Mitoxantrone (Novantrone®, Amgen). Several other immunosuppressive agents are used, although not FDA approved..

Among them, Cladribine, a chlorinated purine analogue 2-chloro-2'deoxyadenosine analogue (2-CdA), has been suggested to be useful in the treatment of MS (*EP 626853B1 and US 5,506,214*).

Several clinical studies with Cladribine in patients with multiple sclerosis have investigated the use of i.v. and s.c. Cladribine in MS.

Two double-blind, placebo controlled Phase II studies were conducted respectively in the treatment of Chronic Progressive MS (*Selby et al., 1998, Can. J. Neurol. Sci., 25:295-299*) and Relapsing-Remitting MS respectively (*Romine et al., 1999, Proceedings of the Association of American Physicians, 111, 1, 35-44*).

In the first trial, the Cladribine dose used was 0.1 mg/kg/day for 7 days by continuous i.v. infusion. The treatment for repeated for 4 consecutive months.

In the second clinical trial, the Cladribine dose used was 0.07mg/kg/day for 5 days by subcutaneous injection. The treatment was repeated for 6 consecutive months.

In addition, placebo controlled Phase III study was conducted in patients with primary progressive (PP) or secondary progressive (SP) multiple sclerosis (*Rice at al., 2000, Neurology, 54, 5, 1145-1155).* In this study, both patient groups received Cladribine by subcutaneous injection at a dose of 0.07 mg/kg/day. The treatment was repeated for either 2 months or 6 months.

The Phase II clinical studies provided evidence for the positive effects of Cladribine in patients with MS in terms of Kutzke Extended Disability Status Scale (EDSS), Scripps Neurologic rating Scale (SNRS) scores and Magnetic Resonance Imaging (MRI) findings (*Beutler et al., 1996, Proc. Nat. Acad. Sci. USA, 93, 1716-1720; Romine et al., 1999 above)*. Phase III study results, were positive on the significant reduction of MRI-measured brain lesions (*Rice at al., 2000, above*).

Some adverse effects (AEs), such as increased incidence of infections related to compromised immune function or myelosuppression, were observed with the highest doses (*Selby et al., 1998, above; Beutler et al., 1994, Acta hematol., 91:10-15*). Due to the narrow margin of safety between the efficacy dose and the dose of occurrence of AEs, to date, all clinical trials for Cladribine in multiple sclerosis have been conducted using either i.v. or s.c. administration. As a result, *Beutler et al. (Beutler et al., 1996, Seminars in Hematology, 33, 1(S1), 45-52)* excluded the oral route for the treatment of multiple sclerosis with Cladribine.

Therefore, it would be desirable to have a method for treating multiple sclerosis comprising the oral administration of Cladribine that would permit the same or improved effect on MS lesions while decreasing the occurrence and/or severity adverse events. In addition, as MS is a chronic disease, it would be desirable to decrease the occurrence and/or severity adverse events in such a way that re-treatments are possible.

Summary of the Invention

The present invention is directed towards a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, wherein the preparation is to be the orally administered. Particularly, the invention is directed towards a use of Cladribine for the preparation of a medicament for the treatment of relapsing-remitting

multiple sclerosis or early secondary progressive multiple sclerosis and wherein re-treatments are possible.

An embodiment of the invention provides an improved dosing regimen for Cladribine in the treatment of multiple sclerosis.

An additional embodiment of the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein adverse effects are reduced, allowing further use of Cladribine.

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein the Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In another embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction treatment wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A maintenance treatment wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i).

Detailed Description of the invention

Definitions

The "total dose" or "cumulative dose" refers to the total dose of Cladribine administered during the treatment, i.e. the dose reached at the end of the treatment that is calculated by adding the daily doses. For example, the total dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days is 3.5 mg/kg.

"The total effective dose" or "cumulative effective dose" refers to the bioavailable dose of Cladribine after a given administration period, i.e. the bioavailable dose reached at the end of the treatment that is calculated by adding the daily doses reduced by the bioavailability coefficient. For example, the total effective dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days wherein the bioavailability of Cladribine is of about 40% is 1.4 mg/kg.

Typically, the bioavailability of Cladribine or of a Cladribine formulation used in the context of this invention is from about 30% to about 90%, preferably from about 40% to about 60%, such as about 50%.

"A week" refers to a period of time of or about 5, about 6 or about 7 days.

"A month" refers to a period of time of or about 28, about 29, about 30 or about 31 days.

"Treatment" comprises the sequential succession of an "induction treatment" and at least a "maintenance treatment". Typically, a treatment according to the invention comprises an "induction treatment" and about one or about two or about three maintenance treatments.

Typically, a treatment according to the invention is of about 2 years (about 24 months) or about 3 years (about 36 months) or about 4 years (about 48 months).

An "Induction Treatment" consists in the sequential succession of **(i)** an induction period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention is orally administered and **(ii)** a Cladribine-free period. An induction period lasts up to about 4 months or up to about 3 month or up to about 2 months. For example, an induction period lasts for about 2 to about 4 months. An induction period consists in the oral administration of Cladribine or a pharmaceutical preparation thereof during about 1 to about 7 days each month.

A "Cladribine-free period" is a period wherein no Cladribine is administered to the patient. During a Cladribine-free period, the patient can be free of any administration or be dosed with a placebo-pill or another drug except. A Cladribine-free period lasts up to about 10 months or up to 9 months or up to about 8 months. For example, a Cladribine-free period lasts from about 8 to about 10 months.

A "Maintenance Treatment" consists in the sequential succession of **(i)** a maintenance period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention is orally administered at a lower dose than the Cladribine dose orally administered during the induction treatment and **(ii)** a Cladribine-free period. A maintenance period lasts for up to about 4 months, or up to about 3 months, or up to about 2 months, preferably up to about 2 months. For example, a maintenance period lasts for about 2 to about 4 months, preferably for about 2 months. A maintenance period consists in the oral administration of Cladribine or of a pharmaceutical preparation thereof during about 1 to about 7 days each month.

Within the context of this invention, the beneficial effect, including but not limited to an attenuation, reduction, decrease or diminishing of the pathological development after onset of the disease, may be seen after one or more a "treatments", after an "induction treatment", after a "maintenance treatment" or during a Cladribine-free period.

"Daily dose" refers to the total dose of Cladribine orally administered to the patient each day of administration. The daily dose can be reached through a single or several administrations per day, such as for example once a day, twice a day or three times a day.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinetic properties, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

Patients suffering from MS can be defined for example as having clinically definite or laboratory-definite MS according to Schumacher or Poser criteria *(Schumacher et al., 1965, Ann. NY Acad. Sci. 1965; 122:552-568; Poser et al., 1983, Ann. Neurol. 13(3): 227-31).*

"Relapses" involve neurologic problems that occur over a short period, typically days but sometimes as short as hours or even minutes. These attacks most often involve motor, sensory, visual or coordination problems early in the disease. Later, bladder, bowel, sexual and cognitive problems may be shown. Sometimes the attack onset occurs over several weeks. Typical MS relapse involves a period of worsening, with development of neurological deficits, then a plateau, in which the patient is not getting any better but also not getting any worse followed by a recovery period. Recovery usually begins within a few weeks.

"Efficacy" of a treatment according to the invention can be measured based on changes in the course of disease in response to a use according to the invention. For example, treatment of MS efficacy can be measured by the frequency of relapses in RRMS and the

presence or absence of new lesions in the CNS as detected using methods such as MRI technique (*Miller et al., 1996, Neurology, 47(Suppl 4): S217; Evans et al., 1997, Ann. Neurology, 41:125-132*).

The observation of the reduction and/or suppression of MRI T_1 gadolinium -enhanced lesions (thought to represent areas of active inflammation) gives a primary efficacy variable.

Secondary efficacy variables include MRI T_1 enhanced brain lesion volume, MRI T_1 enhanced lesion number, MRI T_2 lesion volume (thought to represent total disease burden, i.e. demyelination, gliosis, inflammation and axon loss), MRI T_1 enhanced hypointense lesion volume (thought to represent primarily demyelination and axon loss), time-to-progression of MS, frequency and severity of exacerbations and time-to-exacerbation, Expanded Disability Status Scale score and Scripps Neurologic Rating Scale (SNRS) score (*Sipe et al., 1984, Neurology, 34, 1368-1372*). Methods of early and accurate diagnosis of multiple sclerosis and of following the disease progression are described in *Mattson, 2002, Expert Rev. Neurotherapeutics, 319-328.*

Degree of disability of MS patients can be for example measured by Kurtzke Expanded Disability Status Scale (EDSS) score (*Kurtzke, 1983, Neurology, 33, 14444-1452*). Typically a decrease in EDSS score corresponds to an improvement in the disease and conversely, an increase in EDSS score corresponds to a worsening of the disease.

Cladribine (2-CdA)

2-CdA and its pharmacologically acceptable salts may be used in the practice of this invention.

Cladribine can be formulated in any pharmaceutical preparation suitable for oral administration. Representative oral formulations of 2-CdA are described in (*WO 96/19230; WO 96/19229; US 6,194,395; US 5,506,214; WO 2004/087100; WO 2004/087101*), the

contents of which are incorporated herein by reference. Examples of ingredients for oral formulations are given below.

Processes for preparing 2-CdA are well known in the art. For example, the preparation of 2-CdA is described in (*EP 173,059; WO 04/028462; WO 04/028462; US 5,208,327; WO 00/64918*) and *Robins et al., J. Am. Chem. Soc., 1984, 106: 6379*. Alternatively, pharmaceutical preparations of 2-CdA may be purchased from Bedford Laboratories, Bedford, Ohio.

Oral administration of Cladribine may be in capsule, tablet, oral suspension, or syrup form. The tablet or capsules may contain from about 3 to 500 mg of Cladribine. Preferably they may contain about 3 to about 10 mg of Cladribine, more preferably about 3, about 5 or about 10 mg of Cladribine. The capsules may be gelatin capsules and may contain, in addition to Cladribine in the quantity indicated above, a small quantity, for example less than 5% by weight, magnesium stearate or other excipient. Tablets may contain the foregoing amount of the compound and a binder, which may be a gelatin solution, a starch paste in water, polyvinyl polyvinyl alcohol in water, etc. with a typical sugar coating.

Compositions

Compositions of this invention may further comprise one or more pharmaceutically acceptable additional ingredient(s) such as alum, stabilizers, antimicrobial agents, buffers, coloring agents, flavoring agents, adjuvants, and the like.

Compositions of this invention may be in the form of tablets or lozenges formulated in a conventional manner. For example, tablets and capsules for oral administration may contain conventional excipients including, but not limited to, binding agents, fillers, lubricants, disintegrants and wetting agents. Binding agents include, but are not limited to, syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and polyvinylpyrrolidone. Fillers

include, but are not limited to, lactose, sugar, microcrystalline cellulose, maizestarch, calcium phosphate, and sorbitol. Lubricants include, but are not limited to, magnesium stearate, stearic acid, talc, polyethylene glycol, and silica. Disintegrants include, but are not limited to, potato starch and sodium starch glycollate. Wetting agents include, but are not limited to, sodium lauryl sulfate). Tablets may be coated according to methods well known in the art.

Compositions of this invention may also be liquid formulations including, but not limited to, aqueous or oily suspensions, solutions, emulsions, syrups, and elixirs. The compositions may also be formulated as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain additives including, but not limited to, suspending agents, emulsifying agents, nonaqueous vehicles and preservatives. Suspending agent include, but are not limited to, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents include, but are not limited to, lecithin, sorbitan monooleate, and acacia. Nonaqueous vehicles include, but are not limited to, edible oils, almond oil, fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol. Preservatives include, but are not limited to, methyl or propyl p-hydroxybenzoate and sorbic acid.

Combination

According to the invention, Cladribine can be administered alone or in combination with IFN-beta, prophylactically or therapeutically to an individual prior to, simultaneously or sequentially with other therapeutic regimens or agents (e.g. multiple drug regimens), in a therapeutically effective amount, especially therapeutic agents for the treatment of multiple sclerosis. Active agents that are administered simultaneously with other therapeutic agents can be administered in the same or different compositions and in the same or different routes of administration.

In one embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered during the Cladribine-free period.

In another embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered after the "treatment" according to the invention.

The term "interferon-beta (IFN-β)", as used herein, is intended to include fibroblast interferon in particular of human origin, as obtained by isolation from biological fluids or as obtained by DNA recombinant techniques from prokaryotic or eukaryotic host cells, as well as its salts, functional derivatives, variants, analogs and active fragments.
IFN-β suitable in accordance with the present invention is commercially available e.g. as Rebif® (Serono), Avonex® (Biogen) or Betaferon® (Schering). The use of interferons of human origin is also preferred in accordance with the present invention. The term interferon, as used herein, is intended to encompass salts, functional derivatives, variants, analogs and active fragments thereof.
Rebif® (recombinant human interferon-β) is the latest development in interferon therapy for multiple sclerosis (MS) and represents a significant advance in treatment. Rebif® is interferon (IFN)-beta 1a, produced from mammalian cell lines. It was established that interferon beta-1a given subcutaneously three times per week is efficacious in the treatment of Relapsing-Remitting Multiple Sclerosis (RRMS). Interferon beta-1a can have a positive effect on the long-term course of MS by reducing number and severity of relapses and reducing the burden of the disease and disease activity as measured by MRI.

The dosing of IFN-β in the treatment of relapsing-remitting MS according to the invention depends on the type of IFN-β used.

In accordance with the present invention, where IFN is recombinant IFN-β1b produced in E. Coli, commercially available under the trademark Betaseron®, it may preferably be

administered sub-cutaneously every second day at a dosage of about of 250 to 300 μg or 8 MIU to 9.6 MIU per person.

In accordance with the present invention, where IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Avonex®, it may preferably be administered intra-muscularly once a week at a dosage of about of 30μg to 33 μg or 6 MIU to 6.6 MIU per person.

In accordance with the present invention, when IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Rebif®, it may preferably be administered sub-cutaneously three times a week (TIW) at a dosage of 22 to 44 μg or 6 MIU to 12 MIU per person.

Patients

Patients according to the invention are patients suffering from multiple sclerosis, preferably RRMS or early SPMS.

In an embodiment of the invention, patients are selected from human males or females between 18 and 55 years age.

In another embodiment of the invention, patients had at least one relapse within the prior 12 months of the treatment.

Use according to the invention

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months or up to about 3 months or up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months or up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (ii) period lasts up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period (ii) lasts up to about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (iv) period lasts up to about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein a placebo-pill is administered during the Cladribine-free period.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period is free of any administration.

In another further embodiment, the invention provides a use according to the invention wherein the maintenance period lasts up to about 4 months, or up to about 3 months, or up to about 2 months, preferably up to about 2 months.

In another further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

In a preferred embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i)

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In another embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period lasts up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months; the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In a preferred embodiment, the invention provides Cladribine for use as a medicament for the treatment of multiple sclerosis wherein the medicament is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine about 3 to 30 mg Cladribine, preferably 5 to 20 mg Cladribine, most preferably 10 mg Cladribine.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered once a day during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered several times a day administered once a day during the induction period, preferably twice or three times a day, more preferably twice a day.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 1 to about 7 days per month, preferably from about 5 to about 7 days per month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 0.02 days/kg to about 0.08 days/kg per month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 2 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 3 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 4 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 5 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 6 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to any of the preceding claims wherein the pharmaceutical formulation is to be administered in combination with interferon-beta.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.5 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In another further embodiment, the invention provides a method according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

Examples

The following abbreviations refer respectively to the definitions below:

kg (kilogram), **µg** (microgram), **mg** (milligram), **AEs** (Adverse effects), CNS (Cnetral nervous system), **CSF** (Cerebrospinal fluid), **EDSS** (Expanded Disability Status Scale, **SNRS** (Scripps Neurologic Rating Scale), **IFN** (interferon), **i.v.** (intra -veinous), **MIU** (Million International units), **MS** (multiple sclerosis), **MRI** (Magnetic resonance imaging), **p.o.** (per os), **PPMS** (Primary progressive multiple sclerosis), **PRMS** (Progressive relapsing multiple sclerosis), **RRMS** (Relapsing-remitting multiple sclerosis), **SPMS** (Secondary progressive multiple sclerosis), **s.c.** (subcutaneous), **TIW** (Three times a week), **UI** (International unit).

The efficacy and safety of oral Cladribine administration, eventually multi-dose administration, according to the invention can be assessed for example following the protocol below:

Example 1: Oral cladribine in the treatment of relapsing forms of MS

A study of sixty patients with relapsing forms of clinically definite multiple sclerosis is undertaken. Each patient is first examined for normal hepatic, renal, and bone marrow functioning to establish baseline values.

Patients are selected from Male or Female, between 18 and 55 years of age who had one or more relapses within the prior 12 months. Female patients are non-pregnant female.

Patients are randomly assigned to one of the treatment groups listed in Table 1 below:

Table 1:

Group	2CdA
1	-
2	1.75 mg/kg
3	3.5 mg/kg

Each of the patients in Groups 2 and 3 receives 3 mg or 10 mg 2CdA (1, 2 or 3 administration(s) a day depending on the patient's weight) combined in cyclodextrin formulation as described in WO 2004/087101, Example 3. The Compositions of the Cladribine formulations in 3 mg or 10 mg 2CdA tablets containing hydroxypropyl-beta-cyclodextrin are listed in Table 2 below:

Table 2:

Name of ingredients	Formula mg/tablet	Formula mg/tablet
Cladribine-2-hydroxypropyl-ß-cyclodextrin- complex*	153.75 equivalent to 10 mg 2CdA	30.60 equivalent to 3 mg 2CdA
Sorbitol powder	44.25	68.4
Magnesium Stearate (vegetable grade)	2.0	1.00
Total	200.0	100

* Cladribine is complexed and lyophilised with 2-hydroxypropyl-ß-cyclodextrin as a separate process as described in WO 2004/087101.

Examples of administration schemes for the induction period depending on the patient's weight are given below in Tables 3 and 4 for the target doses of 1.75 mg/kg and 3.5 mg/kg respectively. For the maintenance period, the example of administration scheme of Table 3 is applicable.

Table 3:

Patient weight ranges (kg)			Total target dose (kg) equivalent to 1.75 mg/kg		Number of pills (10 mg)/induction period		
Min	Mid range	Max	Min	Max	Month 1	Month 2	Total
40	42.5	44.9	28	31.4	4	3	7
45	47.5	49.9	31.5	34.9	4	4	8
50	52.5	54.9	35	38.4	5	4	9
55	57.5	59.9	38.5	41.9	5	5	10
60	62.5	64.9	42	45.4	5	5	10
65	67.5	69.9	45.5	48.9	6	5	11
70	72.5	74.9	49	52.4	6	6	12
75	77.5	79.9	52.5	55.9	7	6	13
80	82.5	84.9	56	59.4	7	6	13
85	87.5	89.9	59.5	62.9	7	7	14

Patient weight ranges (kg)			Total target dose (kg) equivalent to 1.75 mg/kg		Number of pills (10 mg)/induction period		
Min	Mid range	Max	Min	Max	Month 1	Month 2	Total
90	92.5	94.9	63	66.4	8	7	15
95	97.5	99.9	66.5	69.9	8	8	16
100	102.5	104.9	70	73.4	9	8	17
105	107.5	109.9	73.5	76.9	9	9	18
110	112.5	114.9	77	80.4	9	9	18
115	117.5	119.9	80.5	83.9	10	9	19

Table 4:

Patient weight ranges (kg)			Total target dose (kg) equivalent to 3.5 mg/kg		Number of pills (10 mg)/induction period				
Min	Mid range	Max	Min	Max	Month 1	Month 2	Month 3	Month 4	Total
40	42.5	44.9	56	62.9	4	4	3	3	14
45	47.5	49.9	63	69.9	4	4	4	4	16
50	52.5	54.9	70	76.9	5	4	4	4	17
55	57.5	59.9	77	83.9	5	5	5	4	19
60	62.5	64.9	84	90.9	6	5	5	5	21
65	67.5	69.9	91	97.9	6	6	5	5	22
70	72.5	74.9	98	104.9	6	6	6	6	24
75	77.5	79.9	105	111.9	7	7	6	6	26
80	82.5	84.9	112	118.9	7	7	7	6	27
85	87.5	89.9	119	125.9	7	7	7	7	28
90	92.5	94.9	126	132.9	8	8	7	7	30
95	97.5	99.9	133	139.9	8	8	8	8	32
100	102.5	104.9	140	146.9	9	8	8	8	33
105	107.5	109.9	147	153.9	9	9	9	8	35
110	112.5	114.9	154	160.9	10	9	9	9	37

Patient weight ranges (kg)			Total target dose (kg) equivalent to 3.5 mg/kg		Number of pills (10 mg)/induction period				
Min	Mid range	Max	Min	Max	Month 1	Month 2	Month 3	Month 4	Total
115	117.5	119.9	161	167.9	10	10	9	9	38

In Group 1 patients receive a placebo (saline) for 4 months followed by 8 months of no treatment.

In Group 2 patients receive a daily oral administration of Cladribine for about 5 days a month during 2 months (induction period) of 2CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg (total dose of about 1.75 mg/kg for a bioavailablility of about 40%); followed by administration of placebo for 2 months; followed by 8 months of no treatment.

In Group 3 patients receive a daily oral administration of Cladribine for about 5 days a month during 4 months (induction period) of 2CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 4 months approximates about 1.4 mg/kg (total dose of about 3.5 mg/kg for a bioavailablility of about 40%); followed by 8 months of no treatment.

Beginning at month 13, all 3 patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 months of no treatment.

Finally, beginning at month 25, all patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 more months of no treatment.

Patients are monitored to determine whether there is any progression or improvement of brain lesions associated with progression of MS through MRI scans and neurological examination as described in *Miller et al., 1996, above; Evans et al., 1997, above*; *Sipe et al., 1984, above*; and *Mattson, 2002, above*. All patients have a baseline and MRI study (brain or spinal cord, according to localization of the lesions) at month 12.
The patient's disability progression and the time for having a first relapse are monitored as well as the proportion of relapse-fee patients at 24 months.

Lymphocyte markers and monocyte counts are monitored in the patients.

Patients in Groups 2 and 3 have a decrease in brain lesions.

The data show that the 2CdA regimen consisting in the succession of an induction treatment and maintenance treatments is efficient in decreasing brain lesions and no severe adverse effect is observed.

Claims:

1. Use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:
   (i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;
   (ii) A Cladribine-free period wherein no Cladribine is administered;
   (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
   (iv) A Cladribine-free period wherein no Cladribine is administered.

2. Use according to claim 1 wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months.

3. Use according to claims 1 or 2 wherein the induction period lasts up to about 2 months.

4. Use according to any of the preceding claims wherein the induction period lasts up to about 4 months.

5. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

6. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg.

7. Use according to any of the preceding claims wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months, or up to about 8 months.

8. Use according to any of the preceding claims wherein the Cladribine-free (iv) period lasts up to about 10 months.

9. Use according to any of the preceding claims wherein the maintenance period lasts up to about 4 months, or up to about 3 months or up to about 2 months.

10. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

11. Use according to claim 1 wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period lasts up to about 4 months, or up to about 3 months or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 8 months or up to about 10 months; the maintenance period lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total

dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

12. Use according to any of the preceding claims wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine about 3 to 30 mg Cladribine.

13. Use according to any of the preceding claims wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine about 10 mg Cladribine.

14. Use according to any of the preceding claims wherein the pharmaceutical formulation is orally administered about 1 to about 7 days per month during the induction period.

15. Use according to any of the preceding claims wherein the steps (iii) to (iv) are repeated at least one or two times.

16. Use according to any of the preceding claims wherein wherein the pharmaceutical formulation is to be administered in combination with interferon-beta.

Abstract of the invention:

The present invention is related to the use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis, wherein the preparation is to be the orally administered and wherein re-treatments are possible.

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By Authority of the
Under Secretary of Commerce for Intellectual Property
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T. Lawrence

T. LAWRENCE
Certifying Officer

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Giampiero	De Luca	Conches, Switzerland

Additional inventors are being named on the _____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Cladribine Regimen for Treating Multiple Sclerosis

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 30
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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.
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Respectfully submitted,

SIGNATURE _____

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

Cladribine regimen for treating Multiple Sclerosis

Field of the Invention

The present invention relates to the use of multiple doses of Cladribine for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis.

Background of the Invention

Multiple sclerosis (MS) is the most known chronic inflammatory demyelinating disease of the central nervous system in humans. The onset of the disease typically occurs during ages 20 to 40. Women are affected approximately twice as often as men.

Over time, MS may result in the accumulation of various neurological disabilities. Clinical disability in MS is presumed to be a result of repeated inflammatory injury with subsequent loss of myelin and axons, leading to tissue atrophy.

MS is manifested in physical symptoms (relapses and disability progression), Central Nervous System (CNS) inflammation, brain atrophy and cognitive impairment. Presenting symptoms include focal sensory deficits, focal weakness, visual problems, imbalance and fatigue. Sexual impairment and sphincter dysfunction may occur. Approximately half of the patients with MS may experience cognitive impairment or depression.

MS is now considered to be a multi-phasic disease and periods of clinical quiescence (remissions) occur between exacerbations. Remissions vary in length and may last several years but are infrequently permanent.

Four courses of the disease are individualized: relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR) multiple sclerosis.

More than 80% of patients with MS will initially display a RR course with clinical exacerbation of neurological symptoms, followed by a recovery that may or may not be complete (*Lublin and Reingold, Neurology, 1996, 46:907-911*).

During RRMS, accumulation of disability results from incomplete recovery from relapses. Approximately, half of the patients with RRMS switch to a progressive course, called SPMS, 10 years after the diseased onset. During the SP phase, worsening of disability results from the accumulation of residual symptoms after exarcerbation but also from insidious progression between exacerbations (*Lublin and Reingold above).* 10% of MS patients have PPMS which is characterized by insidious progression of the symptoms from the disease onset. Less than 5 % of patients have PRMS and are often considered to have the same prognosis as PPMS. It is suggested that distinct pathogenic mechanisms may be involved in different patient sub-groups and have wide-ranging implications for disease classification (*Lassmann et al., 2001, Trends Mol. Med., 7, 115-121; Lucchinetti et al., Curr. Opin. Neurol., 2001, 14, 259-269*).

MS onset is defined by the occurrence of the first neurological symptoms of CNS dysfunction. Advances in cerebrospinal fluid (CSF) analysis and magnetic resonance imaging (MRI) have simplified the diagnostic process and facilitated early diagnostic (*Noseworthy et al., The New England Journal of Medicine, 2000, 343, 13, 938-952*). The International Panel on the Diagnosis of MS issued revised criteria facilitating the diagnosis of MS and including MRI together with clinical and para-clinical diagnostic methods (*Mc Donald et al., 2001, Ann. Neurol., 50:121-127*).

Current medications for MS which are disease modifying treatments, i.e. modifying the course of MS, modulate or suppress the immune system. There are four FDA approved immunomodulating agents for RRMS: three beta interferons (Betaseron®, Berlex; Avonex®, Biogen; Rebif®, Serono) and Glatimarer Acetate (Copaxone®, Amgen). There is also one FDA approved immunosuppressing drug for worsening MS, Mitoxantrone (Novantrone®, Amgen). Several other immunosuppressive agents are used, although not FDA approved.

Among them, Cladribine, a chlorinated purine analogue 2-chloro-2'deoxyadenosine analogue (2-CdA), has been suggested to be useful in the treatment of MS (*EP 626853B1 and US 5,506,214*).

Several clinical studies with Cladribine in patients with multiple sclerosis have investigated the use of i.v. and s.c. Cladribine in MS.

Two double-blind, placebo controlled Phase II studies were conducted respectively in the treatment of Chronic Progressive MS (*Selby et al., 1998, Can. J. Neurol. Sci., 25:295-299*) and Relapsing-Remitting MS respectively (*Romine et al., 1999, Proceedings of the Association of American Physicians, 111, 1, 35-44*).

In the first trial, the Cladribine dose used was 0.1 mg/kg/day for 7 days by continuous i.v. infusion. The treatment for repeated for 4 consecutive months.

In the second clinical trial, the Cladribine dose used was 0.07mg/kg/day for 5 days by subcutaneous injection. The treatment was repeated for 6 consecutive months.

In addition, placebo controlled Phase III study was conducted in patients with primary progressive (PP) or secondary progressive (SP) multiple sclerosis (*Rice at al., 2000, Neurology, 54, 5, 1145-1155).* In this study, both patient groups received Cladribine by subcutaneous injection at a dose of 0.07 mg/kg/day. The treatment was repeated for either 2 months or 6 months.

The Phase II clinical studies provided evidence for the positive effects of Cladribine in patients with MS in terms of Kutzke Extended Disability Status Scale (EDSS), Scripps Neurologic rating Scale (SNRS) scores and Magnetic Resonance Imaging (MRI) findings (*Beutler et al., 1996, Proc. Nat. Acad. Sci. USA, 93, 1716-1720; Romine et al., 1999 above)*. Phase III study results, were positive on the significant reduction of MRI-measured brain lesions (*Rice at al., 2000, above*).

Some adverse effects (AEs), such as increased incidence of infections related to compromised immune function or myelosuppression, were observed with the highest doses (*Selby et al., 1998, above; Beutler et al., 1994, Acta hematol., 91:10-15*). Due to the narrow margin of safety between the efficacy dose and the dose of occurrence of AEs, to date, all clinical trials for Cladribine in multiple sclerosis have been conducted using either i.v. or s.c. administration. As a result, *Beutler et al. (Beutler et al., 1996, Seminars in Hematology, 33, 1(S1), 45-52*) excluded the oral route for the treatment of multiple sclerosis with Cladribine.

Therefore, it would be desirable to have a method for treating multiple sclerosis comprising the oral administration of Cladribine that would permit the same or improved effect on MS lesions while decreasing the occurrence and/or severity adverse events. In addition, as MS is a chronic disease, it would be desirable to decrease the occurrence and/or severity adverse events in such a way that re-treatments are possible.

Summary of the Invention

The present invention is directed towards a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, wherein the preparation is to be the orally administered. Particularly, the invention is directed towards a use of Cladribine for the preparation of a medicament for the treatment of relapsing-remitting

multiple sclerosis or early secondary progressive multiple sclerosis and wherein re-treatments are possible.

An embodiment of the invention provides an improved dosing regimen for Cladribine in the treatment of multiple sclerosis.

An additional embodiment of the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein adverse effects are reduced, allowing further use of Cladribine.

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein the Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In another embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction treatment wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A maintenance treatment wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i).

Detailed Description of the invention

Definitions

The "total dose" or "cumulative dose" refers to the total dose of Cladribine administered during the treatment, i.e. the dose reached at the end of the treatment that is calculated by adding the daily doses. For example, the total dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days is 3.5 mg/kg.

"The total effective dose" or "cumulative effective dose" refers to the bioavailable dose of Cladribine after a given administration period, i.e. the bioavailable dose reached at the end of the treatment that is calculated by adding the daily doses reduced by the bioavailability coefficient. For example, the total effective dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days wherein the bioavailability of Cladribine is of about 40% is 1.4 mg/kg.

Typically, the bioavailability of Cladribine or of a Cladribine formulation used in the context of this invention is from about 30% to about 90%, preferably from about 40% to about 60%, such as about 50%.

"A week" refers to a period of time of or about 5, about 6 or about 7 days.

"A month" refers to a period of time of or about 28, about 29, about 30 or about 31 days.

"Treatment" comprises the sequential succession of an "induction treatment" and at least a "maintenance treatment". Typically, a treatment according to the invention comprises an "induction treatment" and about one or about two or about three maintenance treatments.

Typically, a treatment according to the invention is of about 2 years (about 24 months) or about 3 years (about 36 months) or about 4 years (about 48 months).

An "Induction Treatment" consists in the sequential succession of **(i)** an induction period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention is orally administered and **(ii)** a Cladribine-free period. An induction period lasts up to about 4 months or up to about 3 month or up to about 2 months. For example, an induction period lasts for about 2 to about 4 months. An induction period consists in the oral administration of Cladribine or a pharmaceutical preparation thereof during about 1 to about 7 days each month.

A "Cladribine-free period" is a period wherein no Cladribine is administered to the patient. During a Cladribine-free period, the patient can be free of any administration or be dosed with a placebo-pill or another drug except. A Cladribine-free period lasts up to about 10 months or up to 9 months or up to about 8 months. For example, a Cladribine-free period lasts from about 8 to about 10 months.

A "Maintenance Treatment" consists in the sequential succession of **(i)** a maintenance period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention is orally administered at a lower dose than the Cladribine dose orally administered during the induction treatment and **(ii)** a Cladribine-free period. A maintenance period lasts for up to about 4 months, or up to about 3 months, or up to about 2 months, preferably up to about 2 months. For example, a maintenance period lasts for about 2 to about 4 months, preferably for about 2 months. A maintenance period consists in the oral administration of Cladribine or of a pharmaceutical preparation thereof during about 1 to about 7 days each month.

Within the context of this invention, the beneficial effect, including but not limited to an attenuation, reduction, decrease or diminishing of the pathological development after onset of the disease, may be seen after one or more a "treatments", after an "induction treatment", after a "maintenance treatment" or during a Cladribine-free period.

"Daily dose" refers to the total dose of Cladribine orally administered to the patient each day of administration. The daily dose can be reached through a single or several administrations per day, such as for example once a day, twice a day or three times a day.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinetic properties, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

Patients suffering from MS can be defined for example as having clinically definite or laboratory-definite MS according to Schumacher or Poser criteria *(Schumacher et al., 1965, Ann. NY Acad. Sci. 1965; 122:552-568; Poser et al., 1983, Ann. Neurol. 13(3): 227-31).*

"Relapses" involve neurologic problems that occur over a short period, typically days but sometimes as short as hours or even minutes. These attacks most often involve motor, sensory, visual or coordination problems early in the disease. Later, bladder, bowel, sexual and cognitive problems may be shown. Sometimes the attack onset occurs over several weeks. Typical MS relapse involves a period of worsening, with development of neurological deficits, then a plateau, in which the patient is not getting any better but also not getting any worse followed by a recovery period. Recovery usually begins within a few weeks.

"Efficacy" of a treatment according to the invention can be measured based on changes in the course of disease in response to a use according to the invention. For example, treatment of MS efficacy can be measured by the frequency of relapses in RRMS and the

presence or absence of new lesions in the CNS as detected using methods such as MRI technique (*Miller et al., 1996, Neurology*, 47 (Suppl 4):5217; *Evans et al., 1997, Ann. Neurology, 41:125-132*).

The observation of the reduction and/or suppression of MRI T_1 gadolinium-enhanced lesions (thought to represent areas of active inflammation) gives a primary efficacy variable.

Secondary efficacy variables include MRI T_1 enhanced brain lesion volume, MRI T_1 enhanced lesion number, MRI T_2 lesion volume (thought to represent total disease burden, i.e. demyelination, gliosis, inflammation and axon loss), MRI T_1 enhanced hypointense lesion volume (thought to represent primarily demyelination and axon loss), time-to-progression of MS, frequency and severity of exacerbations and time-to-exacerbation, Expanded Disability Status Scale score and Scripps Neurologic Rating Scale (SNRS) score (*Sipe et al., 1984, Neurology, 34, 1368-1372*). Methods of early and accurate diagnosis of multiple sclerosis and of following the disease progression are described in *Mattson, 2002, Expert Rev. Neurotherapeutics, 319-328.*

Degree of disability of MS patients can be for example measured by Kurtzke Expanded Disability Status Scale (EDSS) score (*Kurtzke, 1983, Neurology, 33, 14444-1452*). Typically a decrease in EDSS score corresponds to an improvement in the disease and conversely, an increase in EDSS score corresponds to a worsening of the disease.

Cladribine (2-CdA)

2-CdA and its pharmacologically acceptable salts may be used in the practice of this invention.

Cladribine can be formulated in any pharmaceutical preparation suitable for oral administration. Representative oral formulations of 2-CdA are described in (*WO 96/19230; WO 96/19229; US 6,194,395; US 5,506,214; WO 2004/087100; WO 2004/087101*), the

contents of which are incorporated herein by reference. Examples of ingredients for oral formulations are given below.

Processes for preparing 2-CdA are well known in the art. For example, the preparation of 2-CdA is described in (*EP 173,059; WO 04/028462; WO 04/028462; US 5,208,327; WO 00/64918*) and *Robins et al., J. Am. Chem. Soc., 1984, 106: 6379.* Alternatively, pharmaceutical preparations of 2-CdA may be purchased from Bedford Laboratories, Bedford, Ohio.

Oral administration of Cladribine may be in capsule, tablet, oral suspension, or syrup form. The tablet or capsules may contain from about 3 to 500 mg of Cladribine. Preferably they may contain about 3 to about 10 mg of Cladribine, more preferably about 3, about 5 or about 10 mg of Cladribine. The capsules may be gelatin capsules and may contain, in addition to Cladribine in the quantity indicated above, a small quantity, for example less than 5% by weight, magnesium stearate or other excipient. Tablets may contain the foregoing amount of the compound and a binder, which may be a gelatin solution, a starch paste in water, polyvinyl polyvinyl alcohol in water, etc. with a typical sugar coating.

Compositions

Compositions of this invention may further comprise one or more pharmaceutically acceptable additional ingredient(s) such as alum, stabilizers, antimicrobial agents, buffers, coloring agents, flavoring agents, adjuvants, and the like.

Compositions of this invention may be in the form of tablets or lozenges formulated in a conventional manner. For example, tablets and capsules for oral administration may contain conventional excipients including, but not limited to, binding agents, fillers, lubricants, disintegrants and wetting agents. Binding agents include, but are not limited to, syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and polyvinylpyrrolidone. Fillers

include, but are not limited to, lactose, sugar, microcrystalline cellulose, maizestarch, calcium phosphate, and sorbitol. Lubricants include, but are not limited to, magnesium stearate, stearic acid, talc, polyethylene glycol, and silica. Disintegrants include, but are not limited to, potato starch and sodium starch glycollate. Wetting agents include, but are not limited to, sodium lauryl sulfate). Tablets may be coated according to methods well known in the art.

Compositions of this invention may also be liquid formulations including, but not limited to, aqueous or oily suspensions, solutions, emulsions, syrups, and elixirs. The compositions may also be formulated as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain additives including, but not limited to, suspending agents, emulsifying agents, nonaqueous vehicles and preservatives. Suspending agent include, but are not limited to, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents include, but are not limited to, lecithin, sorbitan monooleate, and acacia. Nonaqueous vehicles include, but are not limited to, edible oils, almond oil, fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol. Preservatives include, but are not limited to, methyl or propyl p-hydroxybenzoate and sorbic acid.

Combination

According to the invention, Cladribine can be administered alone or in combination with IFN-beta, prophylactically or therapeutically to an individual prior to, simultaneously or sequentially with other therapeutic regimens or agents (e.g. multiple drug regimens), in a therapeutically effective amount, especially therapeutic agents for the treatment of multiple sclerosis. Active agents that are administered simultaneously with other therapeutic agents can be administered in the same or different compositions and in the same or different routes of administration.

In one embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered during the Cladribine-free period.

In another embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered after the "treatment" according to the invention.

The term "interferon-beta (IFN-β)", as used herein, is intended to include fibroblast interferon in particular of human origin, as obtained by isolation from biological fluids or as obtained by DNA recombinant techniques from prokaryotic or eukaryotic host cells, as well as its salts, functional derivatives, variants, analogs and active fragments.
IFN-β suitable in accordance with the present invention is commercially available e.g. as Rebif® (Serono), Avonex® (Biogen) or Betaferon® (Schering). The use of interferons of human origin is also preferred in accordance with the present invention. The term interferon, as used herein, is intended to encompass salts, functional derivatives, variants, analogs and active fragments thereof.
Rebif® (recombinant human interferon-β) is the latest development in interferon therapy for multiple sclerosis (MS) and represents a significant advance in treatment. Rebif® is interferon (IFN)-beta 1a, produced from mammalian cell lines. It was established that interferon beta-1a given subcutaneously three times per week is efficacious in the treatment of Relapsing-Remitting Multiple Sclerosis (RRMS). Interferon beta-1a can have a positive effect on the long-term course of MS by reducing number and severity of relapses and reducing the burden of the disease and disease activity as measured by MRI.

The dosing of IFN-β in the treatment of relapsing-remitting MS according to the invention depends on the type of IFN-β used.

In accordance with the present invention, where IFN is recombinant IFN-β1b produced in E. Coli, commercially available under the trademark Betaseron®, it may preferably be

administered sub-cutaneously every second day at a dosage of about of 250 to 300 μg or 8 MIU to 9.6 MIU per person.

In accordance with the present invention, where IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Avonex®, it may preferably be administered intra-muscularly once a week at a dosage of about of 30μg to 33 μg or 6 MIU to 6.6 MIU per person.

In accordance with the present invention, when IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Rebif®, it may preferably be administered sub-cutaneously three times a week (TIW) at a dosage of 22 to 44 μg or 6 MIU to 12 MIU per person.

Patients

Patients according to the invention are patients suffering from multiple sclerosis, preferably RRMS or early SPMS.

In an embodiment of the invention, patients are selected from human males or females between 18 and 55 years age.

In another embodiment of the invention, patients had at least one relapse within the prior 12 months of the treatment.

Use according to the invention

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months or up to about 3 months or up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months or up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (ii) period lasts up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period (ii) lasts up to about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (iv) period lasts up to about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein a placebo-pill is administered during the Cladribine-free period.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period is free of any administration.

In another further embodiment, the invention provides a use according to the invention wherein the maintenance period lasts up to about 4 months, or up to about 3 months, or up to about 2 months, preferably up to about 2 months.

In another further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

In a preferred embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i)

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In another embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period lasts up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months; the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In a preferred embodiment, the invention provides Cladribine for use as a medicament for the treatment of multiple sclerosis wherein the medicament is to be orally administered following the sequential steps below:

(v) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(vi) A Cladribine-free period wherein no Cladribine is administered;

(vii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i)

(viii) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine about 3 to 30 mg Cladribine, preferably 5 to 20 mg Cladribine, most preferably 10 mg Cladribine.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered once a day during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered several times a day administered once a day during the induction period, preferably twice or three times a day, more preferably twice a day.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 1 to about 7 days per month, preferably from about 5 to about 7 days per month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 0.02 days/kg to about 0.08 days/kg per month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 2 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 3 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 4 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 5 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 6 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to any of the preceding claims wherein the pharmaceutical formulation is to be administered in combination with interferon-beta.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.5 mg/kg to about 3.5 mg/kg;

(v) A Cladribine-free period wherein no Cladribine is administered;

(vi) A maintenance period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(vii) A Cladribine-free period wherein no Cladribine is administered.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In another further embodiment, the invention provides a method according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

Examples

The following abbreviations refer respectively to the definitions below:

kg (kilogram), **µg** (microgram), **mg** (milligram), **AEs** (Adverse effects), CNS (Cnetral nervous system), **CSF** (Cerebrospinal fluid), **EDSS** (Expanded Disability Status Scale, **SNRS** (Scripps Neurologic Rating Scale), **IFN** (interferon), **i.v.** (intra-veinous), **MIU** (Million International units), **MS** (multiple sclerosis), **MRI** (Magnetic resonance imaging), **p.o.** (per os), **PPMS** (Primary progressive multiple sclerosis), **PRMS** (Progressive relapsing multiple sclerosis), **RRMS** (Relapsing-remitting multiple sclerosis), **SPMS** (Secondary progressive multiple sclerosis), **s.c.** (subcutaneous), **TIW** (Three times a week), **UI** (International unit).

The efficacy and safety of oral Cladribine administration, eventually multi-dose administration, according to the invention can be assessed for example following the protocol below:

Example 1: Oral cladribine in the treatment of relapsing forms of MS

A study of sixty patients with relapsing forms of clinically definite multiple sclerosis is undertaken. Each patient is first examined for normal hepatic, renal, and bone marrow functioning to establish baseline values.

Patients are selected from Male or Female, between 18 and 55 years of age who had one or more relapses within the prior 12 months. Female patients are non-pregnant female.

Patients are randomly assigned to one of the treatment groups listed in Table 1 below:

Table 1:

Group	2CdA
1	-
2	1.75 mg/kg
3	3.5 mg/kg

Each of the patients in Groups 2 and 3 receives 3 mg or 10 mg 2CdA (1, 2 or 3 administration(s) a day depending on the patient's weight) combined in cyclodextrin formulation as described in WO 2004/087101, Example 3. The Compositions of the Cladribine formulations in 3 mg or 10 mg 2CdA tablets containing hydroxypropyl-beta-cyclodextrin are listed in Table 2 below:

Table 2:

Name of ingredients	Formula mg/tablet	Formula mg/tablet
Cladribine-2-hydroxypropyl-ß-cyclodextrin- complex*	153.75 equivalent to 10 mg 2CdA	30.60 equivalent to 3 mg 2CdA
Sorbitol powder	44.25	68.4
Magnesium Stearate (vegetable grade)	2.0	1.00
Total	200.0	100

* Cladribine is complexed and lyophilised with 2-hydroxypropyl-ß-cyclodextrin as a separate process as described in WO 2004/087101.

Examples of administration schemes for the induction period depending on the patient's weight are given below in Tables 3 and 4 for the target doses of 1.75 mg/kg and 3.5 mg/kg respectively. For the maintenance period, the example of administration scheme of Table 3 is applicable.

Table 3:

Patient weight ranges (kg)			Total target dose (kg) equivalent to 1.75 mg/kg		Number of pills (10 mg)/induction period		
Min	Mid range	Max	Min	Max	Month 1	Month 2	Total
40	42.5	44.9	28	31.4	4	3	7
45	47.5	49.9	31.5	34.9	4	4	8
50	52.5	54.9	35	38.4	5	4	9
55	57.5	59.9	38.5	41.9	5	5	10
60	62.5	64.9	42	45.4	5	5	10
65	67.5	69.9	45.5	48.9	6	5	11
70	72.5	74.9	49	52.4	6	6	12
75	77.5	79.9	52.5	55.9	7	6	13
80	82.5	84.9	56	59.4	7	6	13
85	87.5	89.9	59.5	62.9	7	7	14

Patient weight ranges (kg)			Total target dose (kg) equivalent to 1.75 mg/kg		Number of pills (10 mg)/induction period		
Min	Mid range	Max	Min	Max	Month 1	Month 2	Total
90	92.5	94.9	63	66.4	8	7	15
95	97.5	99.9	66.5	69.9	8	8	16
100	102.5	104.9	70	73.4	9	8	17
105	107.5	109.9	73.5	76.9	9	9	18
110	112.5	114.9	77	80.4	9	9	18
115	117.5	119.9	80.5	83.9	10	9	19

Table 4:

Patient weight ranges (kg)			Total target dose (kg) equivalent to 3.5 mg/kg		Number of pills (10 mg)/induction period				
Min	Mid range	Max	Min	Max	Month 1	Month 2	Month 3	Month 4	Total
40	42.5	44.9	56	62.9	4	4	3	3	14
45	47.5	49.9	63	69.9	4	4	4	4	16
50	52.5	54.9	70	76.9	5	4	4	4	17
55	57.5	59.9	77	83.9	5	5	5	4	19
60	62.5	64.9	84	90.9	6	5	5	5	21
65	67.5	69.9	91	97.9	6	6	5	5	22
70	72.5	74.9	98	104.9	6	6	6	6	24
75	77.5	79.9	105	111.9	7	7	6	6	26
80	82.5	84.9	112	118.9	7	7	7	6	27
85	87.5	89.9	119	125.9	7	7	7	7	28
90	92.5	94.9	126	132.9	8	8	7	7	30
95	97.5	99.9	133	139.9	8	8	8	8	32
100	102.5	104.9	140	146.9	9	8	8	8	33
105	107.5	109.9	147	153.9	9	9	9	8	35
110	112.5	114.9	154	160.9	10	9	9	9	37

Patient weight ranges (kg)			Total target dose (kg) equivalent to 3.5 mg/kg		Number of pills (10 mg)/induction period				
Min	Mid range	Max	Min	Max	Month 1	Month 2	Month 3	Month 4	Total
115	117.5	119.9	161	167.9	10	10	9	9	38

In Group 1 patients receive a placebo (saline) for 4 months followed by 8 months of no treatment.

In Group 2 patients receive a daily oral administration of Cladribine for about 5 days a month during 2 months (induction period) of 2CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg (total dose of about 1.75 mg/kg for a bioavailablility of about 40%); followed by administration of placebo for 2 months; followed by 8 months of no treatment.

In Group 3 patients receive a daily oral administration of Cladribine for about 5 days a month during 4 months (induction period) of 2CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 4 months approximates about 1.4 mg/kg (total dose of about 3.5 mg/kg for a bioavailablility of about 40%); followed by 8 months of no treatment.

Beginning at month 13, all 3 patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 months of no treatment.

Finally, beginning at month 25, all patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 more months of no treatment.

Patients are monitored to determine whether there is any progression or improvement of brain lesions associated with progression of MS through MRI scans and neurological examination as described in *Miller et al., 1996, above; Evans et al., 1997, above*; *Sipe et al., 1984, above*; and *Mattson, 2002, above*. All patients have a baseline and MRI study (brain or spinal cord, according to localization of the lesions) at month 12.
The patient's disability progression and the time for having a first relapse are monitored as well as the proportion of relapse-fee patients at 24 months.

Lymphocyte markers and monocyte counts are monitored in the patients.

Patients in Groups 2 and 3 have a decrease in brain lesions.

The data show that the 2CdA regimen consisting in the succession of an induction treatment and maintenance treatments is efficient in decreasing brain lesions and no severe adverse effect is observed.

Claims:

1. Method of treating multiple sclerosis, with 2-CdA wherein, 2-CdA is orally administered following the sequential steps below:
   (i) Administering 2-CdA, such that the total dose of 2-CdA reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;
   (ii) Administering no 2-CdA during a Cladribine free period;
   (iii) Administering 2-CdA such that the total dose of 2-CdA reached at the end of a maintenance period is lower than the total dose of 2-CdA reached at the end of the induction period (i);
   (iv) And optionally, a Cladribine-free period wherein no 2-CdA is administered.

2. The method of claim 1, wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months.

3. The method of claim 1, wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

4. The method of claim 1, wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months, or up to about 8 months.

5. The method of claim 1, wherein the maintenance period lasts up to about 4 months, or up to about 3 months or up to about 2 months.

6. The method of claim 1, wherein the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

7. The method of claim 1, wherein the maintenance period is followed by a Cladribine-free period.

8. The method of claim 1, comprising the following:

(i) An induction period wherein 2-CdA is administered and wherein the total dose of 2-CdA reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no 2-CdA is administered;

(iii) A maintenance period wherein the total dose of 2-CdA reached at the end of the maintenance period is lower than the total dose of 2-CdA reached at the end of the induction period (i); and,

(iv) Optionally a Cladribine-free period wherein no 2-CdA is administered.

wherein the induction period lasts up to about 4 months, or up to about 3 months or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 8 months or up to about 10 months; the maintenance period lasts up to about 2 months; the optional Cladribine-free period lasts up to about 10 months; the total dose of 2-CdA reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

9. The method of claim 1 to 8, wherein 2-CdA is to be orally administered at a daily dose of about 3 to about 30 mg.

10. The method of claim 1 to 8, wherein 2-CdA is to be orally administered at a daily dose of about 10 mg.

11. The method of claim 1 to 8, wherein 2-CdA is orally administered about 1 to about 7 days per month during the induction period.

12. The method of claim 1 to 8, wherein the steps (iii) are repeated at least one or two times.

13. The method of claim 1 to 8, wherein 2-CdA is to be administered in combination with interferon-beta.

Abstract of the invention:

The present invention is related to the use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis, wherein the preparation is to be the orally administered and wherein re-treatments are possible.

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(54) Title: CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

(57) Abstract: The present invention is related to the use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis, wherein the preparation is to be the orally administered and wherein re-treatments are possible.

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Cladribine regimen for treating Multiple Sclerosis

Field of the Invention

The present invention relates to the use of multiple doses of Cladribine for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis.

Background of the Invention

Multiple sclerosis (MS) is the most known chronic inflammatory demyelinating disease of the central nervous system in humans. The onset of the disease typically occurs during ages 20 to 40. Women are affected approximately twice as often as men.

Over time, MS may result in the accumulation of various neurological disabilities. Clinical disability in MS is presumed to be a result of repeated inflammatory injury with subsequent loss of myelin and axons, leading to tissue atrophy.

MS is manifested in physical symptoms (relapses and disability progression), Central Nervous System (CNS) inflammation, brain atrophy and cognitive impairment. Presenting symptoms include focal sensory deficits, focal weakness, visual problems, imbalance and fatigue. Sexual impairment and sphincter dysfunction may occur. Approximately half of the patients with MS may experience cognitive impairment or depression.

MS is now considered to be a multi-phasic disease and periods of clinical quiescence (remissions) occur between exacerbations. Remissions vary in length and may last several years but are infrequently permanent.

Four courses of the disease are individualized: relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR) multiple sclerosis.

More than 80% of patients with MS will initially display a RR course with clinical exacerbation of neurological symptoms, followed by a recovery that may or may not be complete (*Lublin and Reingold, Neurology, 1996, 46:907-911*).

During RRMS, accumulation of disability results from incomplete recovery from relapses. Approximately, half of the patients with RRMS switch to a progressive course, called SPMS, 10 years after the diseased onset. During the SP phase, worsening of disability results from the accumulation of residual symptoms after exarcerbation but also from insidious progression between exacerbations (*Lublin and Reingold above).* 10% of MS patients have PPMS which is characterized by insidious progression of the symptoms from the disease onset. Less than 5 % of patients have PRMS and are often considered to have the same prognosis as PPMS. It is suggested that distinct pathogenic mechanisms may be involved in different patient sub-groups and have wide-ranging implications for disease classification (*Lassmann et al., 2001, Trends Mol. Med., 7, 115-121; Lucchinetti et al., Curr. Opin. Neurol., 2001, 14, 259-269*).

MS onset is defined by the occurrence of the first neurological symptoms of CNS dysfunction. Advances in cerebrospinal fluid (CSF) analysis and magnetic resonance imaging (MRI) have simplified the diagnostic process and facilitated early diagnostic (*Noseworthy et al., The New England Journal of Medicine, 2000, 343, 13, 938-952*). The International Panel on the Diagnosis of MS issued revised criteria facilitating the diagnosis of MS and including MRI together with clinical and para-clinical diagnostic methods (*Mc Donald et al., 2001, Ann. Neurol., 50:121-127*).

Current medications for MS which are disease modifying treatments, i.e. modifying the course of MS, modulate or suppress the immune system. There are four FDA approved immunomodulating agents for RRMS: three beta interferons (Betaseron®, Berlex; Avonex®, Biogen; Rebif®, Serono) and Glatimarer Acetate (Copaxone®, Amgen). There is also one FDA approved immunosuppressing drug for worsening MS, Mitoxantrone

(Novantrone®, Amgen). Several other immunosuppressive agents are used, although not FDA approved.

Among them, Cladribine, a chlorinated purine analogue 2-chloro-2'deoxyadenosine analogue (2-CdA), has been suggested to be useful in the treatment of MS (*EP 626853B1 and US 5,506,214*).

Several clinical studies with Cladribine in patients with multiple sclerosis have investigated the use of i.v. and s.c. Cladribine in MS.

Two double-blind, placebo controlled Phase II studies were conducted respectively in the treatment of Chronic Progressive MS (*Selby et al., 1998, Can. J. Neurol. Sci., 25:295-299*) and Relapsing-Remitting MS respectively (*Romine et al., 1999, Proceedings of the Association of American Physicians, 111, 1, 35-44*).

In the first trial, the Cladribine dose used was 0.1 mg/kg/day for 7 days by continuous i.v. infusion. The treatment for repeated for 4 consecutive months.

In the second clinical trial, the Cladribine dose used was 0.07mg/kg/day for 5 days by subcutaneous injection. The treatment was repeated for 6 consecutive months.

In addition, placebo controlled Phase III study was conducted in patients with primary progressive (PP) or secondary progressive (SP) multiple sclerosis (*Rice at al., 2000, Neurology, 54, 5, 1145-1155).* In this study, both patient groups received Cladribine by subcutaneous injection at a dose of 0.07 mg/kg/day. The treatment was repeated for either 2 months or 6 months.

The Phase II clinical studies provided evidence for the positive effects of Cladribine in patients with MS in terms of Kutzke Extended Disability Status Scale (EDSS), Scripps Neurologic rating Scale (SNRS) scores and Magnetic Resonance Imaging (MRI) findings (*Beutler et al., 1996, Proc. Nat. Acad. Sci. USA, 93, 1716-1720; Romine et al., 1999 above).* Phase III study results, were positive on the significant reduction of MRI-measured brain lesions (*Rice at al., 2000, above*).

Some adverse effects (AEs), such as increased incidence of infections related to compromised immune function or myelosuppression, were observed with the highest doses (*Selby et al., 1998, above; Beutler et al., 1994, Acta hematol., 91:10-15*). Due to the narrow margin of safety between the efficacy dose and the dose of occurrence of AEs, to date, all clinical trials for Cladribine in multiple sclerosis have been conducted using either i.v. or s.c. administration. As a result, *Beutler et al. (Beutler et al., 1996, Seminars in Hematology, 33, 1(S1), 45-52*) excluded the oral route for the treatment of multiple sclerosis with Cladribine.

Grieb et al. reported a small trial in 11 patients with remitting-relapsing multiple sclerosis (*Grieb et al., 1995, Archivum Immunologiae et Therapiae Experimentalis, 43 (5-6), 323-327*) wherein Cladribine has been orally administered during 6 monthly courses of 5 days at a total dose of about 4-5.7 mg/kg (patients of about 52 and about 75 kilos, respectively) i.e. a total effective dose of 2-2.85 mg/kg. For some patients, a single re-treatment of 5 days was performed at a cumulative dose of 0.4-0.66 mg/kg after a cladribine free-period of 3 or 6 months. The side effects observed with the regimen above were said to be less severe than the ones observed in the study on patients suffering from chronic progressive multiple sclerosis treated by i.v. infusion of Cladribine (*Sipe et al., 1994, Lancet, 344, 9-13*) but were still present. In addition, the therapeutic efficacy of the oral regimen above versus the i.v. infusion therapy was questioned (*Grieb et al., 1995, above*) and a group of "non-responders" has been identified (*Stelmasiak et al., 1998, Laboratory Investigations, 4(1), 4-8*).

Therefore, it would be desirable to have a method for treating multiple sclerosis comprising the oral administration of Cladribine that would permit the same or improved effect on MS lesions while decreasing the occurrence and/or severity adverse events. In addition, as MS is a chronic disease, it would be desirable to decrease the occurrence and/or severity adverse events in such a way that re-treatments are possible. A sustained benefit of Cladribine treatment between the treatment periods is also desirable.

Summary of the Invention

The present invention is directed towards a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, wherein the preparation is to be the orally administered. Particularly, the invention is directed towards a use of Cladribine for the preparation of a medicament for the treatment of relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis and wherein re-treatments are possible.

An embodiment of the invention provides an improved dosing regimen for Cladribine in the treatment of multiple sclerosis.

An additional embodiment of the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein adverse effects are reduced, allowing further use of Cladribine.

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein the Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In another embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction treatment wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance treatment wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

Detailed Description of the invention

Definitions

The "total dose" or "cumulative dose" refers to the total dose of Cladribine administered during the treatment, i.e. the dose reached at the end of the treatment that is calculated by adding the daily doses. For example, the total dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days is 3.5 mg/kg or the total dose of Cladribine corresponding to a treatment of 0.35 mg/kg Cladribine per day during 5 days is 1.7 mg/kg.

"The total effective dose" or "cumulative effective dose" refers to the bioavailable dose of Cladribine after a given administration period, i.e. the bioavailable dose reached at the end of the treatment that is calculated by adding the daily doses reduced by the bioavailability coefficient. For example, the total effective dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days wherein the bioavailability of Cladribine is of about 40% is 1.4 mg/kg or the total effective dose of Cladribine corresponding to a treatment of 0.35 mg/kg Cladribine per day during 5 days wherein the bioavailability of Cladribine is of about 40% is 0.7 mg/kg.

Typically, the bioavailability of Cladribine or of a Cladribine formulation used in the context of this invention is from about 30% to about 90%, preferably from about 40% to about 60%, such as about 50%.

"A week" refers to a period of time of or about 5, about 6 or about 7 days.

"A month" refers to a period of time of or about 28, about 29, about 30 or about 31 days.

"Treatment" comprises the sequential succession of an "induction treatment" and at least a "maintenance treatment". Typically, a treatment according to the invention comprises an "induction treatment" and about one or about two or about three maintenance treatments. Typically, a treatment according to the invention is of about 2 years (about 24 months) or about 3 years (about 36 months) or about 4 years (about 48 months).

An "Induction Treatment" consists in the sequential succession of **(i)** an induction period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention is orally administered and **(ii)** a Cladribine-free period. An induction period lasts up to about 4 months or up to about 3 month or up to about 2 months. For example, an induction period lasts for about 2 to about 4 months. An induction period consists in the oral administration of Cladribine or a pharmaceutical preparation thereof during about 1 to about 7 days each month.

A "Cladribine-free period" is a period wherein no Cladribine is administered to the patient. During a Cladribine-free period, the patient can be free of any administration or be dosed with a placebo-pill or another drug except. A Cladribine-free period lasts up to about 10 months or up to 9 months or up to about 8 months. For example, a Cladribine-free period lasts from about 8 to about 10 months, typically at least of about 8 months.

A "Maintenance Treatment" consists in the sequential succession of **(i)** a maintenance period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention is orally administered at a lower dose than the Cladribine dose orally administered during

the induction treatment and **(ii)** a Cladribine-free period. A maintenance period lasts for up to about 4 months, or up to about 3 months, or up to about 2 months, preferably up to about 2 months. For example, a maintenance period lasts for about 2 to about 4 months, preferably for about 2 months. A maintenance period consists in the oral administration of Cladribine or of a pharmaceutical preparation thereof during about 1 to about 7 days each month.

Within the context of this invention, the beneficial effect, including but not limited to an attenuation, reduction, decrease or diminishing of the pathological development after onset of the disease, may be seen after one or more a "treatments", after an "induction treatment", after a "maintenance treatment" or during a Cladribine-free period.

"Daily dose" refers to the total dose of Cladribine orally administered to the patient each day of administration. The daily dose can be reached through a single or several administrations per day, such as for example once a day, twice a day or three times a day.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinctic properties, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.
Patients suffering from MS can be defined for example as having clinically definite or laboratory-definite MS according to Schumacher or Poser criteria *(Schumacher et al., 1965, Ann. NY Acad. Sci. 1965; 122:552-568; Poser et al., 1983, Ann. Neurol. 13(3): 227-31).*

"Relapses" involve neurologic problems that occur over a short period, typically days but sometimes as short as hours or even minutes. These attacks most often involve motor, sensory, visual or coordination problems early in the disease. Later, bladder, bowel, sexual and cognitive problems may be shown. Sometimes the attack onset occurs over several weeks. Typical MS relapse involves a period of worsening, with development of neurological deficits, then a plateau, in which the patient is not getting any better but also

not getting any worse followed by a recovery period. Recovery usually begins within a few weeks.

"Efficacy" of a treatment according to the invention can be measured based on changes in the course of disease in response to a use according to the invention. For example, treatment of MS efficacy can be measured by the frequency of relapses in RRMS and the presence or absence of new lesions in the CNS as detected using methods such as MRI technique (*Miller et al., 1996, Neurology, 47(Suppl 4): S217; Evans et al., 1997, Ann. Neurology, 41:125-132*).

The observation of the reduction and/or suppression of MRI T_1 gadolinium-enhanced lesions (thought to represent areas of active inflammation) gives a primary efficacy variable.

Secondary efficacy variables include MRI T_1 enhanced brain lesion volume, MRI T_1 enhanced lesion number, MRI T_2 lesion volume (thought to represent total disease burden, i.e. demyelination, gliosis, inflammation and axon loss), MRI T_1 enhanced hypointense lesion volume (thought to represent primarily demyelination and axon loss), time-to-progression of MS, frequency and severity of exacerbations and time-to-exacerbation, Expanded Disability Status Scale score and Scripps Neurologic Rating Scale (SNRS) score (*Sipe et al., 1984, Neurology, 34, 1368-1372*). Methods of early and accurate diagnosis of multiple sclerosis and of following the disease progression are described in *Mattson, 2002, Expert Rev. Neurotherapeutics, 319-328.*

Degree of disability of MS patients can be for example measured by Kurtzke Expanded Disability Status Scale (EDSS) score (*Kurtzke, 1983, Neurology, 33, 1444-1452*). Typically a decrease in EDSS score corresponds to an improvement in the disease and conversely, an increase in EDSS score corresponds to a worsening of the disease.

Cladribine (2-CdA)

2-CdA and its pharmacologically acceptable salts may be used in the practice of this invention.

Cladribine can be formulated in any pharmaceutical preparation suitable for oral administration. Representative oral formulations of 2-CdA are described in (*WO 96/19230; WO 96/19229; US 6,194,395; US 5,506,214; WO 2004/087100; WO 2004/087101*), the contents of which are incorporated herein by reference. Examples of ingredients for oral formulations are given below.

Processes for preparing 2-CdA are well known in the art. For example, the preparation of 2-CdA is described in (*EP 173,059; WO 04/028462; WO 04/028462; US 5,208,327; WO 00/64918*) and *Robins et al., J. Am. Chem. Soc., 1984, 106: 6379.* Alternatively, pharmaceutical preparations of 2-CdA may be purchased from Bedford Laboratories, Bedford, Ohio.

Oral administration of Cladribine may be in capsule, tablet, oral suspension, or syrup form. The tablet or capsules may contain from about 3 to 500 mg of Cladribine. Preferably they may contain about 3 to about 10 mg of Cladribine, more preferably about 3, about 5 or about 10 mg of Cladribine. The capsules may be gelatin capsules and may contain, in addition to Cladribine in the quantity indicated above, a small quantity, for example less than 5% by weight, magnesium stearate or other excipient. Tablets may contain the foregoing amount of the compound and a binder, which may be a gelatin solution, a starch paste in water, polyvinyl polyvinyl alcohol in water, etc. with a typical sugar coating.

Compositions

Compositions of this invention may further comprise one or more pharmaceutically acceptable additional ingredient(s) such as alum, stabilizers, antimicrobial agents, buffers, coloring agents, flavoring agents, adjuvants, and the like.

Compositions of this invention may be in the form of tablets or lozenges formulated in a conventional manner. For example, tablets and capsules for oral administration may contain conventional excipients including, but not limited to, binding agents, fillers, lubricants,

disintegrants and wetting agents. Binding agents include, but are not limited to, syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and polyvinylpyrrolidone. Fillers include, but are not limited to, lactose, sugar, microcrystalline cellulose, maizestarch, calcium phosphate, and sorbitol. Lubricants include, but are not limited to, magnesium stearate, stearic acid, talc, polyethylene glycol, and silica. Disintegrants include, but are not limited to, potato starch and sodium starch glycollate. Wetting agents include, but are not limited to, sodium lauryl sulfate). Tablets may be coated according to methods well known in the art.

Compositions of this invention may also be liquid formulations including, but not limited to, aqueous or oily suspensions, solutions, emulsions, syrups, and elixirs. The compositions may also be formulated as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain additives including, but not limited to, suspending agents, emulsifying agents, nonaqueous vehicles and preservatives. Suspending agent include, but are not limited to, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents include, but are not limited to, lecithin, sorbitan monooleate, and acacia. Nonaqueous vehicles include, but are not limited to, edible oils, almond oil, fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol. Preservatives include, but are not limited to, methyl or propyl p-hydroxybenzoate and sorbic acid.

Combination

According to the invention, Cladribine can be administered alone or in combination with IFN-beta, prophylactically or therapeutically to an individual prior to, simultaneously or sequentially with other therapeutic regimens or agents (e.g. multiple drug regimens), in a therapeutically effective amount, especially therapeutic agents for the treatment of multiple sclerosis. Active agents that are administered simultaneously with other therapeutic agents can be administered in the same or different compositions and in the same or different routes of administration.

In one embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered during the Cladribine-free period.

In another embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered after the "treatment" according to the invention.

The term "interferon-beta (IFN-β)", as used herein, is intended to include fibroblast interferon in particular of human origin, as obtained by isolation from biological fluids or as obtained by DNA recombinant techniques from prokaryotic or eukaryotic host cells, as well as its salts, functional derivatives, variants, analogs and active fragments.
IFN-β suitable in accordance with the present invention is commercially available e.g. as Rebif® (Serono), Avonex® (Biogen) or Betaferon® (Schering). The use of interferons of human origin is also preferred in accordance with the present invention. The term interferon, as used herein, is intended to encompass salts, functional derivatives, variants, analogs and active fragments thereof.
Rebif® (recombinant human interferon-β) is the latest development in interferon therapy for multiple sclerosis (MS) and represents a significant advance in treatment. Rebif® is interferon (IFN)-beta 1a, produced from mammalian cell lines. It was established that interferon beta-1a given subcutaneously three times per week is efficacious in the treatment of Relapsing-Remitting Multiple Sclerosis (RRMS). Interferon beta-1a can have a positive effect on the long-term course of MS by reducing number and severity of relapses and reducing the burden of the disease and disease activity as measured by MRI.

The dosing of IFN-β in the treatment of relapsing-remitting MS according to the invention depends on the type of IFN-β used.

In accordance with the present invention, where IFN is recombinant IFN-β1b produced in E. Coli, commercially available under the trademark Betaseron®, it may preferably be

administered sub-cutaneously every second day at a dosage of about of 250 to 300 μg or 8 MIU to 9.6 MIU per person.

In accordance with the present invention, where IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Avonex®, it may preferably be administered intra-muscularly once a week at a dosage of about of 30μg to 33 μg or 6 MIU to 6.6 MIU per person.

In accordance with the present invention, when IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Rebif®, it may preferably be administered sub-cutaneously three times a week (TIW) at a dosage of 22 to 44 μg or 6 MIU to 12 MIU per person.

Patients

Patients according to the invention are patients suffering from multiple sclerosis, preferably RRMS or early SPMS.

In an embodiment of the invention, patients are selected from human males or females between 18 and 55 years age.

In another embodiment of the invention, patients had at least one relapse within the prior 12 months of the treatment.

Use according to the invention

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months or up to about 3 months or up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months or up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (ii) period lasts up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (ii) period lasts at least about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period (ii) lasts up to about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (iv) period lasts up to about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (iv) period lasts at least about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free periods (ii) and/or (iv) last between about 8 and about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein a placebo-pill is administered during the Cladribine-free period.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period is free of any administration.

In another further embodiment, the invention provides a use according to the invention wherein the maintenance period lasts up to about 4 months, or up to about 3 months, or up to about 2 months, preferably up to about 2 months.

In another further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the maintenance period (iii) is about 1.7 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

In a preferred embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i)

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period (iii) lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In another embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period (iii) is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period (iii) lasts up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months; the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In a preferred embodiment, the invention provides Cladribine for use as a medicament for the treatment of multiple sclerosis wherein the medicament is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period (iii) lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In another embodiment, the invention provides a a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine about 3 to 30 mg Cladribine, preferably 5 to 20 mg Cladribine, most preferably 10 mg Cladribine.

In another further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the total effective dose of Cladribine reached at the end of the induction period is about 1.4 mg/kg and the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered once a day during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered several times a day administered once a day during the induction period, preferably twice or three times a day, more preferably twice a day.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 1 to about 7 days per month, preferably from about 5 to about 7 days per month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 0.02 days/kg to about 0.08 days/kg per month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 0.02 days/kg to about 0.08 days/kg per month during the maintenance period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 2 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily

dose of about 10 mg Cladribine from day 1 to about day 3 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 4 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 5 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 6 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 4 each month during the induction period and wherein the pharmaceutical formulation is a pharmaceutical formulation described in WO 2004/087101 or in WO 2004/087100.

In another embodiment, the invention provides a use of Cladribine according to any of the preceding claims wherein the pharmaceutical formulation is to be administered in combination with interferon-beta.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.5 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In another further embodiment, the invention provides a method according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

In a preferred embodiment, the invention provides a method of treating multiple sclerosis with Cladribine, wherein Cladribine is orally administered following the sequential steps below:

(i) Administering Cladribine, such that the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) Administering no Cladribine during a Cladribine free period;

(iii) Administering Cladribine such that the total dose of Cladribine reached at the end of a maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) And optionally, a Cladribine-free period wherein no Cladribine is administered.

In a further preferred embodiment, the invention provides a method wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months.

In a further preferred embodiment, the invention provides a method wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the total effective dose of Cladribine reached at the end of the induction period is about 1.4 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months, or up to about 8 months.

In a further preferred embodiment, the invention provides a method wherein the maintenance period lasts up to about 4 months, or up to about 3 months or up to about 2 months.

In a further preferred embodiment, the invention provides a method wherein the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the maintenance period is followed by a Cladribine-free period.

In another further embodiment, the invention provides a method according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

In another further embodiment, the invention provides a method according to the invention wherein the total effective dose of Cladribine reached at the end of the induction period is about 1.4 mg/kg and the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be orally administered at a daily dose of about 3 to about 30 mg.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be orally administered at a daily dose of about 10 mg.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is orally administered about 1 to about 7 days per month during the induction period.

In another further embodiment, the invention provides a method according to the invention wherein the steps (iii) are repeated at least one or two times.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be administered in combination with interferon-beta.

Examples

The following abbreviations refer respectively to the definitions below:

kg (kilogram), **µg** (microgram), **mg** (milligram), **AEs** (Adverse effects), CNS (Cnetral nervous system), **CSF** (Cerebrospinal fluid), **EDSS** (Expanded Disability Status Scale, **SNRS** (Scripps Neurologic Rating Scale), **IFN** (interferon), **i.v.** (intra-veinous), **MIU** (Million International units), **MS** (multiple sclerosis), **MRI** (Magnetic resonance imaging), **p.o.** (per os), **PPMS** (Primary progressive multiple sclerosis), **PRMS** (Progressive relapsing multiple sclerosis), **RRMS** (Relapsing-remitting multiple sclerosis), **SPMS** (Secondary progressive multiple sclerosis), **s.c.** (subcutaneous), **TIW** (Three times a week), **2-CdA** (2-chloro-2'deoxyadenosine or Cladribine), **UI** (International unit).

The efficacy and safety of oral Cladribine administration, eventually multi-dose administration, according to the invention can be assessed for example following the protocol below:

Example 1: Oral cladribine in the treatment of relapsing forms of MS

A study of sixty patients with relapsing forms of clinically definite multiple sclerosis is undertaken. Each patient is first examined for normal hepatic, renal, and bone marrow functioning to establish baseline values.

Patients are selected from Male or Female, between 18 and 55 years of age who had one or more relapses within the prior 12 months. Female patients are non-pregnant female.

Patients are randomly assigned to one of the treatment groups listed in Table 1 below:

Table 1:

Group	2-CdA
1	-
2	1.75 mg/kg
3	3.5 mg/kg

Each of the patients in Groups 2 and 3 receives 3 mg or 10 mg 2-CdA (1, 2 or 3 administration(s) a day depending on the patient's weight) combined in cyclodextrin formulation as described in WO 2004/087101, Example 3. The Compositions of the Cladribine formulations in 3 mg or 10 mg 2-CdA tablets containing hydroxypropyl-beta-cyclodextrin are listed in Table 2 below:

Table 2:

Name of ingredients	**Formula mg/tablet**	**Formula mg/tablet**
Cladribine-2-hydroxypropyl-ß-cyclodextrin- complex*	153.75 equivalent to 10 mg 2-CdA	30.60 equivalent to 3 mg 2-CdA
Sorbitol powder	44.25	68.4
Magnesium Stearate (vegetable grade)	2.0	1.00
Total	200.0	100

* Cladribine is complexed and lyophilised with 2-hydroxypropyl-ß-cyclodextrin as a separate process as described in WO 2004/087101.

Examples of administration schemes for the induction period depending on the patient's weight are given below in Tables 3 and 4 for the target doses of 1.75 mg/kg and 3.5 mg/kg respectively. For the maintenance period, the example of administration scheme of Table 3 is applicable.

Table 3:

Patient weight ranges (kg)			Total target dose (kg) equivalent to 1.75 mg/kg		Number of pills (10 mg)/induction period		
Min	Mid range	Max	Min	Max	Month 1	Month 2	Total
40	42.5	44.9	28	31.4	4	3	7
45	47.5	49.9	31.5	34.9	4	4	8
50	52.5	54.9	35	38.4	5	4	9
55	57.5	59.9	38.5	41.9	5	5	10
60	62.5	64.9	42	45.4	5	5	10
65	67.5	69.9	45.5	48.9	6	5	11
70	72.5	74.9	49	52.4	6	6	12
75	77.5	79.9	52.5	55.9	7	6	13
80	82.5	84.9	56	59.4	7	6	13
85	87.5	89.9	59.5	62.9	7	7	14
90	92.5	94.9	63	66.4	8	7	15
95	97.5	99.9	66.5	69.9	8	8	16
100	102.5	104.9	70	73.4	9	8	17
105	107.5	109.9	73.5	76.9	9	9	18
110	112.5	114.9	77	80.4	9	9	18
115	117.5	119.9	80.5	83.9	10	9	19

Table 4:

Patient weight ranges (kg)			Total target dose (kg) equivalent to 3.5 mg/kg		Number of pills (10 mg)/induction period				
Min	Mid range	Max	Min	Max	Month 1	Month 2	Month 3	Month 4	Total
40	42.5	44.9	56	62.9	4	4	3	3	14
45	47.5	49.9	63	69.9	4	4	4	4	16
50	52.5	54.9	70	76.9	5	4	4	4	17
55	57.5	59.9	77	83.9	5	5	5	4	19
60	62.5	64.9	84	90.9	6	5	5	5	21
65	67.5	69.9	91	97.9	6	6	5	5	22
70	72.5	74.9	98	104.9	6	6	6	6	24

Patient weight ranges (kg)			Total target dose (kg) equivalent to 3.5 mg/kg		Number of pills (10 mg)/induction period				
Min	Mid range	Max	Min	Max	Month 1	Month 2	Month 3	Month 4	Total
75	77.5	79.9	105	111.9	7	7	6	6	26
80	82.5	84.9	112	118.9	7	7	7	6	27
85	87.5	89.9	119	125.9	7	7	7	7	28
90	92.5	94.9	126	132.9	8	8	7	7	30
95	97.5	99.9	133	139.9	8	8	8	8	32
100	102.5	104.9	140	146.9	9	8	8	8	33
105	107.5	109.9	147	153.9	9	9	9	8	35
110	112.5	114.9	154	160.9	10	9	9	9	37
115	117.5	119.9	161	167.9	10	10	9	9	38

In Group 1 patients receive a placebo (saline) for 4 months followed by 8 months of no treatment.

In Group 2 patients receive a daily oral administration of Cladribine for about 5 days a month during 2 months (induction period) of 2-CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg (total dose of about 1.75 mg/kg for a bioavailablility of about 40%); followed by administration of placebo for 2 months; followed by 8 months of no treatment.

In Group 3 patients receive a daily oral administration of Cladribine for about 5 days a month during 4 months (induction period) of 2-CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 4 months approximates about 1.4 mg/kg (total dose of about 3.5 mg/kg for a bioavailablility of about 40%); followed by 8 months of no treatment.

Beginning at month 13, all 3 patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with

the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 months of no treatment.

Finally, beginning at month 25, all patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 more months of no treatment.

Patients are monitored to determine whether there is any progression or improvement of brain lesions associated with progression of MS through MRI scans and neurological examination as described in *Miller et al., 1996, above; Evans et al., 1997, above*; *Sipe et al., 1984, above*; and *Mattson, 2002, above*. All patients have a baseline and MRI study (brain or spinal cord, according to localization of the lesions) at month 12.
The patient's disability progression and the time for having a first relapse are monitored as well as the proportion of relapse-fee patients at 24 months.

Lymphocyte markers and monocyte counts are monitored in the patients.

Patients in Groups 2 and 3 have a decrease in brain lesions.

The data show that the 2-CdA regimen consisting in the succession of an induction treatment and maintenance treatments is efficient in decreasing brain lesions and no severe adverse effect is observed.

Claims:

1. Use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:
   (i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from 1.7 mg/kg to 3.5 mg/kg;
   (ii) A Cladribine-free period wherein no Cladribine is administered;
   (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
   (iv) A Cladribine-free period wherein no Cladribine is administered.

2. Use according to claim 1 wherein the induction period lasts up to 4 months, or up to 3 months, or up to 2 months.

3. Use according to claims 1 or 2 wherein the induction period lasts up to 2 months.

4. Use according to any of the preceding claims wherein the induction period lasts up to 4 months.

5. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the induction period is 1.7 mg/kg.

6. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the induction period is 3.5 mg/kg.

7. Use according to any of the preceding claims wherein the Cladribine-free period lasts up to 10 months, or up to 9 months, or up to 8 months.

8. Use according to any of the preceding claims wherein the Cladribine-free (iv) period lasts up to 10 months.

9. Use according to any of the preceding claims wherein the maintenance period lasts up to 4 months, or up to 3 months or up to 2 months.

10. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the maintenance period is 1.7 mg/kg.

11. Use according to claim 1 wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from 1.7 mg/kg to 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period lasts up to 4 months, or up to 3 months or up to 2 months; the Cladribine-free period (ii) lasts up to 10 months, or up to 8 months or up to 10 months; the maintenance period (iii) lasts up to 2 months; the Cladribine-free period (iv) lasts up to 10 months; the total dose of Cladribine reached at the end of the maintenance period is 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

12. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the induction period is 3.5 mg/kg and the total dose of Cladribine reached at the end of the maintenance period is 1.7 mg/kg.

13. Use according to any of the preceding claims wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine 3 to 30 mg Cladribine.

14. Use according to any of the preceding claims wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine 10 mg Cladribine.

15. Use according to any of the preceding claims wherein the pharmaceutical formulation is orally administered 1 to 7 days per month during the induction period.

16. Use according to any of the preceding claims wherein the steps (iii) to (iv) are repeated at least one or two times.

17. Use according to any of the preceding claims wherein the pharmaceutical formulation is to be administered in combination with interferon-beta.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/056954

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7076 A61K38/21 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, SCISEARCH, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRIEB PAWEL ET AL: "Effect of repeated treatments with cladribine (2-chlorodeoxyadenosine) on blood counts in multiple sclerosis patients" ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS, vol. 43, no. 5-6, 1995, pages 323-327, XP008047072 ISSN: 0004-069X cited in the application abstract page 324, column 1, paragraph 3 page 326, column 2, paragraph 3 ----- -/--	1,7-9, 13-16

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 May 2006	15/05/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cielen, E

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STELMASIAK Z ET AL: "A pilot trial of cladribine (2-chlorodeoxyadenosine) in remitting- relapsing multiple sclerosis" MEDICAL SCIENCE MONITOR 1998 POLAND, vol. 4, no. 1, 1998, pages 4-8, XP008047060 ISSN: 1234-1010 cited in the application abstract page 5, column 1, paragraph 2 - column 2, paragraph 1 page 7, column 1, paragraph 3	1,7-9, 13-16
X	WO 2004/087101 A (IVAX CORPORATION; BODOR, NICHOLAS, S; DANDIKER, YOGESH) 14 October 2004 (2004-10-14) cited in the application page 5, lines 1-7 page 12, lines 4-12 page 23, lines 15-28 page 24, lines 10-26 page 33, line 24 - page 34, line 2; example 4 page 36, line 17 - page 37, line 2; example 5 claims 25,28,36,39	1,2,4, 7-9, 13-15,17
X	LANGTRY H D ET AL: "Cladribine: A review of its use in multiple sclerosis" BIODRUGS 1998 NEW ZEALAND, vol. 9, no. 5, 1998, pages 419-433, XP008047073 ISSN: 1173-8804	1,7-9, 13-16
A	page 422, column 1, paragraph 3 - page 423, column 1, paragraph 1 page 423, column 2, paragraphs 1,2 page 424, column 1, paragraph 5 table III page 430, column 1, paragraph 3 table IV	17
A	ELLISON GEORGE W ET AL: "Oral cladribine for multiple sclerosis" NEUROLOGY, vol. 48, no. 3 SUPPL. 2, 1997, pages A174-A175, XP008047069 & 49TH ANNUAL MEETING OF THE AMERICAN ACADEMY OF NEUROLOGY; BOSTON, MASSACHUSETTS, USA; APRIL 12-19, 1997 ISSN: 0028-3878 the whole document	1-15

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/056954

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 626 853 A (THE SCRIPPS RESEARCH INSTITUTE) 7 December 1994 (1994-12-07) cited in the application page 3, paragraph 23 page 4, paragraphs 25,30 page 8, paragraphs 62-66,71-73 page 8, paragraph 74 - page 9, paragraph 75 page 9, paragraph 78	1-16

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2005/056954

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 2004087101 A	14-10-2004	AU	2004226437 A1	14-10-2004
		BR	PI0408848 A	04-04-2006
		CA	2520523 A1	14-10-2004
		EP	1608344 A2	28-12-2005
EP 0626853 A	07-12-1994	AT	192045 T	15-05-2000
		AU	682818 B2	23-10-1997
		AU	3724993 A	13-09-1993
		BR	9305907 A	21-10-1997
		CA	2130275 A1	02-09-1993
		CH	684310 A5	31-08-1994
		DE	69328474 D1	31-05-2000
		DE	69328474 T2	28-09-2000
		DK	626853 T3	07-08-2000
		FI	943805 A	19-10-1994
		HU	68030 A2	29-05-1995
		JP	2688113 B2	08-12-1997
		JP	7507540 T	24-08-1995
		NO	942765 A	13-09-1994
		NO	20000762 A	13-09-1994
		RU	2130308 C1	20-05-1999
		WO	9316706 A1	02-09-1993

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U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
11/722,018	GIAMPIERO DE LUCA	SER-125

23557
SALIWANCHIK LLOYD & SALIWANCHIK
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INTERNATIONAL APPLICATION NO.	
PCT/EP2005/056954	
I.A. FILING DATE	PRIORITY DATE
12/20/2005	12/22/2004

CONFIRMATION NO. 5532
371 FORMALITIES LETTER

Date Mailed: 11/26/2008

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Filing Date Granted

Applicant is given **TWO MONTHS FROM THE DATE OF THIS NOTICE** within which to comply with the sequence rules, 37 CFR §§ 1.821-1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 CFR § 1.821(g). Extension of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR § 1.136. In no case may an applicant extend the period for response beyond the six-month statutory period. Direct the response to: Mail Stop Missing Parts, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450.

- This application clearly fails to comply with the requirements of 37 CFR. 1.821-1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing", an initial paper or compact disc copy of the "Sequence Listing", **as well as an amendment specifically directing its entry into the application**. Applicant must also provide a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.
- A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing" and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

For questions regarding compliance to 37 CFR 1.821-1.825 requirements, please contact:

- **For Rules Interpretation, call (571) 272-0951**
- **For Patentin Software Program Help, call Patent EBC at 1-866-217-9197 or directly at 703-305-3028 / 703-308-6845 between the hours of 6 a.m. and 12 midnight, Monday through Friday, EST.**
- **Send e-mail correspondence for Patentin Software Program Help @ ebc@uspto.gov**

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
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VONDA M WALLACE

Telephone: (703) 308-9140 EXT 225

PATENT APPLICATION FEE DETERMINATION RECORD

Effective December 8, 2004

Application or Docket Number: 11/722,018

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
U.S. NATIONAL STAGE FEES		
BASIC FEE		
EXAMINATION FEE		
SEARCH FEE		
FEE FOR EXTRA SPEC. PGS.	minus 100 =	/ 50 =
TOTAL CHARGEABLE CLAIMS	20 minus 20 =	
INDEPENDENT CLAIMS	1 minus 3 =	
MULTIPLE DEPENDENT CLAIM PRESENT		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY		OR	LARGE ENTITY	
RATE	FEE		RATE	FEE
BASIC FEE		OR	BASIC FEE	300
EXAM. FEE			EXAM. FEE	200
SEARCH FEE			SEARCH FEE	400
X $ 125 =			X $ 250 =	
X $ 25 =		OR	X $ 50 =	
X $ 100 =		OR	X $ 200 =	
+ $ 180 =		OR	+ $ 360 =	
TOTAL		OR	TOTAL	900

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X $ 25 =		OR	X $ 50 =	
X $ 100 =		OR	X $ 200 =	
+ $ 180 =		OR	+ $ 360 =	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X $ 25 =		OR	X $ 50 =	
X $ 100 =		OR	X $ 200 =	
+ $ 180 =		OR	+ $ 360 =	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than '20', enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than '3', enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

(FOR USE WITH FORM PTO-875)

SERIAL NO.	FILING DATE
11/732,018	
APPLICANT(S)	

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
1	1		1			
2		1				
3		2				
4		3				
5		4				
6		5				
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48						
49						
50						
TOTAL IND.	1		1			
TOTAL DEP.	17		19			
TOTAL CLAIMS	18		20			

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
51						
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93						
94						
95						
96						
97						
98						
99						
100						
TOTAL IND.						
TOTAL DEP.						
TOTAL CLAIMS						

I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on December 10, 2008.

Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

Patent Application
Docket No. SER.125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Giampiero De Luca
Serial No.	:	11/722,018
Filed	:	June 18, 2007
Conf. No.	:	5532
For	:	Cladribine Regimen for Treating Multiple Sclerosis

Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

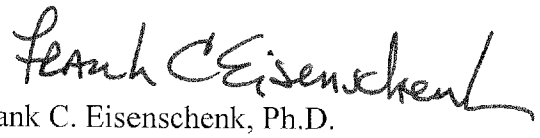
RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Sir:

A Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures was received in the above-referenced patent application dated November 26, 2008. The Notice indicates that the subject application does not comply with the sequence requirements of 37 CFR §§1.821-1.825. Applicant's undersigned representative respectfully submits that the as-filed specification does not contain any sequences. Accordingly, a sequence listing on paper and in computer readable format is not required. However, if the Patent Office believes that there are sequences contained in the subject specification, Applicant respectfully requests that the next communication provide the page and line number.

Applicants believe that no fees are due in connection with filing this Response. However, should the Patent Office determine that fees are due, the Commissioner is authorized to charge any fees as required under 37 CFR §§1.16 or 1.17 to Deposit Account No. 19-0065.

Respectfully submitted,



Frank C. Eisenschenk, Ph.D.
Patent Attorney
Registration No. 45,332
Telephone No.: (352) 375-8100
Facsimile No.: (352) 372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

FCE/sl

J:\SER\125\PTO-Misc\Resp-Not2Comply.doc\DNB/sl

Electronic Acknowledgement Receipt

EFS ID:	4431481
Application Number:	11722018
International Application Number:	
Confirmation Number:	5532
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	GIAMPIERO DE LUCA
Customer Number:	23557
Filer:	Frank Christopher Eisenschenk/Sherry Loke
Filer Authorized By:	Frank Christopher Eisenschenk
Attorney Docket Number:	SER-125
Receipt Date:	10-DEC-2008
Filing Date:	
Time Stamp:	16:19:15
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Response to Pre-Exam Sequence Notice	Resp-Not2Comply.pdf	107558 e60f33fd153135e97baa1f48d060f17706927560	no	2

Warnings:	
Information:	

Total Files Size (in bytes):	107558

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
11/722,018	Giampiero De Luca	SER-125

23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO BOX 142950
GAINESVILLE, FL 32614-2950

INTERNATIONAL APPLICATION NO.	
PCT/EP2005/056954	
I.A. FILING DATE	PRIORITY DATE
12/20/2005	12/22/2004

CONFIRMATION NO. 5532
371 ACCEPTANCE LETTER

OC000000033563838

Date Mailed: 12/12/2008

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as a Designated / Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above and the relevant dates are:

06/18/2007	06/22/2007
DATE OF RECEIPT OF 35 U.S.C. 371(c)(1), (c)(2) and (c)(4) REQUIREMENTS	DATE OF COMPLETION OF ALL 35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE " FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1), (c)(2) and (c)(4) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

The following items have been received:

- Copy of the International Application filed on 06/18/2007
- Copy of the International Search Report filed on 06/18/2007
- Preliminary Amendments filed on 06/18/2007
- Information Disclosure Statements filed on 06/18/2007
- Oath or Declaration filed on 06/18/2007
- U.S. Basic National Fees filed on 06/18/2007
- Priority Documents filed on 06/18/2007
- Power of Attorney filed on 08/16/2007
- Specification filed on 06/18/2007
- Claims filed on 06/18/2007
- Abstracts filed on 06/18/2007

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

VONDA M WALLACE

Telephone: (703) 308-9140 EXT 225

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
11/722,018	Giampiero De Luca	SER-125

23557
SALIWANCHIK LLOYD & SALIWANCHIK
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PO BOX 142950
GAINESVILLE, FL 32614-2950

INTERNATIONAL APPLICATION NO.	
PCT/EP2005/056954	
I.A. FILING DATE	PRIORITY DATE
12/20/2005	12/22/2004

CONFIRMATION NO. 5532
371 WITHDRAWAL NOTICE

OC000000033563814

Date Mailed: 12/12/2008

Letter Regarding a New Notice and/or the Status of the Application

If a new notice or Filing Receipt is enclosed, applicant may disregard the previous notice mailed on 11/26/2008. The time period for reply runs from the mail date of the new notice. Within the time period for reply, applicant is required to file a reply in compliance with the requirements set forth in the new notice to avoid abandonment of the application.

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web. https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If the reply is not filed electronically via EFS-Web, the reply must be accompanied by a copy of the new notice.

If the Office previously granted a petition to withdraw the holding of abandonment or a petition to revive under 37 CFR 1.137, the status of the application has been returned to pending status.

VONDA M WALLACE

Telephone: (703) 308-9140 EXT 225

FORM PCT/DO/EO/WITH (371 Withdrawal Notice)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
11/722,018	06/18/2007	1614	900	SER-125	20	1

CONFIRMATION NO. 5532

23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO BOX 142950
GAINESVILLE, FL 32614-2950

FILING RECEIPT

OC000000033563837

Date Mailed: 12/12/2008

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Giampiero De Luca, Conches, SWITZERLAND;

Assignment For Published Patent Application

Laboratoires Serono S.A., Aubonne, SWITZERLAND

Power of Attorney: The patent practitioners associated with Customer Number 23557

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/EP2005/056954 12/20/2005
which claims benefit of 60/638,669 12/22/2004

Foreign Applications

EUROPEAN PATENT OFFICE (EPO) 04106909.7 12/22/2004

If Required, Foreign Filing License Granted: 12/10/2008

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 11/722,018**

Projected Publication Date: 03/26/2009

Non-Publication Request: No

Early Publication Request: No

Title

CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

Preliminary Class

514

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
11/722,018	06/18/2007	Giampiero De Luca	SER-125

CONFIRMATION NO. 5532

23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO Box 142950
GAINESVILLE, FL 32614

PUBLICATION NOTICE

OC000000035186128

Title:CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

Publication No.US-2009-0081163-A1
Publication Date:03/26/2009

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
11/722,018	06/18/2007	

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☒ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☒ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☒ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT 06/18/2007	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 20	Minus	** 20	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	* 1	Minus	***3	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT 06/18/2007	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 20	Minus	** 20	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/GLORIA NORRIS/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/722,018	06/18/2007	Giampiero De Luca	SER-125	5532

23557 7590 08/03/2009

SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO Box 142950
GAINESVILLE, FL 32614

EXAMINER	
BALLARD, KIMBERLY	
ART UNIT	PAPER NUMBER
1649	

MAIL DATE	DELIVERY MODE
08/03/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	11/722,018	DE LUCA, GIAMPIERO	
	Examiner	Art Unit	
	Kimberly Ballard	1649	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 December 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *18-37* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *18-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *06/18/2007*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Formal Matters

1. Claims 1-17 have been canceled and new claims 18-37 have been added as requested in the preliminary amendment filed on June 18, 2007.

2. Claims **18-37** are pending and under examination in the current office action.

Information Disclosure Statement

3. The information disclosure statement (IDS) filed June 18, 2007 has been considered and is of record.

Claim Objections

4. Claim 30 is objected to because of the following informalities: in step (iv) the claim recites that "the cladribine-free period (ii) lasts up to 10 months, or up to 8 months *or up to 10 months*" (emphasis added), wherein the phrase "up to 10 months" has been unnecessarily repeated. Appropriate correction is required.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

6. Claims 18-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 2004/087101 A2 by Bodor et al. (published October 14, 2004; priority to March 28, 2003; listed on IDS) and US Patent 5,506,214 to Beutler (issued April 9, 1996), in view of US Patent 4,964,848 to Bloom (issued October 23, 1990).

The claims recite a method of treating multiple sclerosis comprising the oral administration of a formulation comprising cladribine, wherein the formulation is administered following the steps of: (i) an induction period wherein the total dose of cladribine reached at the end of this period is from 1.7 mg/kg to 3.5 mg/kg; (ii) a cladribine-free period wherein no formulation is administered; (iii) a maintenance period wherein said formulation is administered and wherein the total dose of cladribine reached at the end of this period is lower than the total dose of cladribine reached at the end of the induction period (i); and (iv) a cladribine-free period wherein no cladribine formulation is administered.

The teachings of Bodor et al. and Beutler are cumulative. Both references teach the use of cladribine for the treatment of multiple sclerosis. Specifically, Bodor discloses treatment of multiple sclerosis comprising administering to a patient in need

thereof a therapeutically effect amount of a composition comprising cladribine, wherein the composition is formulated for oral administration (see, for example, pages 5 and 22). In particular, Bodor teaches that for the treatment of multiple sclerosis, 10 mg of cladribine in solid dosage form is to be administered orally once per day for a period of five to seven days in the first month, repeated for another period of five to seven days in the second month, followed by ten months of no treatment. Alternatively, the patient may be treated with 10 mg of cladribine in the dosage form once per day for a period of five to seven days per month for a total of six months, followed by eighteen months of no treatment (see page 23, lines 7-24). Further, Bodor discloses that one of skill in the art will appreciate that the therapeutically effective amount of cladribine administered may be lowered or increased by fine tuning and/or by administering cladribine with another active ingredient (see page 24, lines 1-4).

Hence, the teachings of Bodor et al. address the following recited limitations of the instant claims: an induction period lasting up to 4 months, or up to 3 months, or up to 2 months (claims 19-23 and 30); the cladribine-free period (step iv) lasts up to 10 months (claims 26, 27 and 30); the maintenance period lasts up to 4 months, or up to 3 months, or up to 2 months (claims 28 and 30); the formulation is to be orally administered at a daily dose of 3 to 30 mg cladribine (claim 32) or at a daily dose of 10 mg cladribine (claim 33); and the pharmaceutical formulation is administered 1 to 7 days per month during the induction period (claim 34).

Finally, regarding claims 36 and 37, Bodor discloses administration of cladribine in conjunction with the administration of one or more additional active ingredients. For

example, in the treatment of multiple sclerosis, Bodor teaches co-administration of interferon beta (see page 24, lines 10-18).

In accord with the teachings of Bodor et al., Beutler discloses a method of treating multiple sclerosis by administration of 2-chloro-2'-deoxyadenosine (a.k.a. cladribine) (see abstract), wherein oral administration is a preferred mode of administration (see column 10, lines 47-48). Beutler teaches that for oral administration, a therapeutically effective daily dose can range from about 0.04 mg/kg to about 1.0 mg/kg/day (see column 10, lines 53-54). Typical administration lasts for a time period of about 5 to about 14 days, with a 7-day time course being usual. Courses (cycles) of administration can also be repeated at monthly intervals. Oral unit dosages can be administered at intervals of one to several days to provide the therapeutically effective dose (see column 11, lines 1-15).

Thus, for an average adult human weighing 150 lbs (68 kg), according to the claimed invention, if the total dose reached at the end of the induction period is 3.5 mg/kg and the total dose reached at the end of the maintenance period is 1.7 mg/kg, for example, this would amount to 238 mg and 115.6 mg cladribine, respectively. According to Beutler, a 68 kg adult receiving oral cladribine therapy for 7 days, for example, could receive a total dose of between 19.04 and 476 mg cladribine, which encompasses the instantly recited doses of claims 18, 24, 25, and 29-31.

Thus, Beutler notes, *in vivo* administration of the above dosages over a time period of about 5 to about 14 days or at weekly or day intervals provides an amount sufficient to kill at least 50 percent of the originally present monocytes (which acts to

down-regulate the autoimmune aspect of MS) (see column 11, lines 1-15). Beutler further teaches that the daily administration course can be repeated periodically over a period of several months, e.g. about three to about nine months. In usual practice, this means that treatments are administered over a period of about 5-7 days and are repeated at about 3 to about 4 week intervals for several months, e.g. about 3 to about 9 months (see column 11, lines 37-50). Therefore, for a 7 day administration period (i.e., the induction period), the total dose of cladribine reached at the end of the 7 days would be in the range of 0.28 to 7.0 mg/kg, which would encompass the instantly recited range of 1.7 to 3.5 mg/kg. And in addition to addressing presently claimed limitations regarding the frequency of the administration cycles, Beutler's disclosure also addresses instantly recited limitations of claims 30 and 35, which state that the maintenance (iii) step and cladribine-free period (iv) step are repeated at least one, two or three times.

Taken together, the combined teachings of Bodor et al. and Beutler provide for method of treating multiple sclerosis comprising oral administration of cladribine, wherein a typical treatment course comprises the daily administration of cladribine for 1-7 days (i.e., induction phase) followed by a cladribine-free period and then another course of daily cladribine for 1-7 days in a subsequent treatment such as the second month (i.e., maintenance phase), followed by up to 10 months of no treatment, wherein a weekly treatment course can be repeated periodically for several months. However, neither of the above references teach that the total dose of cladribine reached at the

end of the maintenance phase is lower than the total dose reached at the end of the induction phase.

Bloom discusses the treatment of multiple sclerosis and other autoimmune diseases, wherein effective treatment requires an intense induction phase lasting from about five to seven weeks during which time lymphocytes are continuously depleted from circulation to a level of less than 500 cells/μl, followed by a maintenance phase in a lower intensity treatment regimen is employed to hold the overall blood lymphocyte count to less than 500 cells/μl (see columns 3-4). While the initial induction phase of treatment is accomplished here by lymphoctapheresis and the maintenance phase is accomplished with the use of immunosuppressive or immunomodulatory agents other than cladribine, the principle of Bloom's teachings remains the same: effective treatment of multiple sclerosis requires an intense induction phase with substantial depletion of blood lymphocytes followed by a more moderate maintenance phase to hold the cell numbers down. Even with respect to the immunomodulatory agents employed in the maintenance phase, Bloom teaches that treatment dosages are lowered with each successive use of the drug (see, for example, column 4, lines 39-49 regarding the use of azathioprine (AZA) and prednisone.

Thus, as evidenced by the prior art, the skilled artisan would have known that effective treatment of multiple sclerosis involves the removal of the majority (e.g., up to 90%; see Beutler, column 11) of activated lymphocytes from the patient in an initial phase of the treatment, followed by a maintenance phase in which the numbers of lymphocytes are maintained at a reduced level. The artisan would have also been

aware that cladribine is useful for the treatment of multiple sclerosis, as cladribine is an immunosuppressive agent that functions by killing activated lymphocytes (and in particular, monocytes, which are presumably activated against self-antigens). Particularly in view of the chronic nature of multiple sclerosis, in order to reduce the severity or duration of future relapses (or to prevent them altogether) the artisan would have been aware that in order for long-term treatment to be successful, it must be sustainable and well-tolerated by the patient.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to treat multiple sclerosis with oral cladribine according to a cyclic treatment regimen, wherein treatment involves an induction phase and a maintenance phase, and wherein the total dosage administered in the maintenance phase is less than the total dosage administered in the induction phase. Additionally, even though cladribine therapy is generally well-tolerated with a low incidence of adverse effects (see, for example, Examples 1 and 2 in the Beutler patent), in order to reduce overall treatment costs associated with cladribine therapy and further lessen the risk of negative side effects, the artisan would have been motivated to use a lower dose in the maintenance phase that is still sufficient to sustain a therapeutically-effective immunosuppressive state. Regardless, each of the recited doses, treatment durations, and frequencies are clearly result effective parameters that a person of ordinary skill in the art would routinely optimize (see MPEP § 2144.05). Optimization of parameters is a routine practice that would be obvious for a person of ordinary skill in the art to employ. Indeed, both Bodor et al. and Beutler assert that it is within the level and skill of the

artisan to fine-tune cladribine dosages and treatment protocols in order to achieve a desired therapeutic effect. Thus, absent some demonstration of unexpected results from the claimed parameters, this optimization would have been obvious at the time of applicant's invention. Furthermore, as was noted by the United States Supreme Court, if a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one method (e.g., Bloom's method of high dose, no dose, low dose), and a person of ordinary skill would recognize that it would improve similar methods (e.g., Bodor et al. or Beutler) in the same way, using the technique is obvious unless its actual application is beyond his or her skill. *KSR,* 127 S. Ct. at 1740. "When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product is not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show it was obvious under 35 U.S.C. 103." *KSR Int'l Co. v. Teleflex Inc.,* 127 S.Ct. 1727, 1742, 82USPQ2d 1385, 1396 (2007).

Conclusion

7. No claims are allowed.

Advisory Information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kimberly Ballard whose telephone number is 571-272-2150. The examiner can normally be reached on Monday-Friday 8:30 AM - 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Stucker can be reached on 571-272-0911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Kimberly Ballard
Art Unit 1649

/Elizabeth C. Kemmerer/
Elizabeth C. Kemmerer, Ph.D.
Primary Examiner, Art Unit 1646

Notice of References Cited	Application/Control No. 11/722,018	Applicant(s)/Patent Under Reexamination DE LUCA, GIAMPIERO	
	Examiner Kimberly Ballard	Art Unit 1649	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-4,964,848	10-1990	Bloom, Philip M.	604/6.03
*	B	US-5,506,214	04-1996	Beutler, Ernest	514/46
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
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Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11722018	DE LUCA, GIAMPIERO
	Examiner	Art Unit
	Kimberly Ballard	1649

SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
Inventor search (PALM, EAST, NPL)	07/28/2009	KAB
EAST (USPAT, USOCR, PGPUB, DERWENT, FPRS, EPO, JPO)	07/28/2009	KAB
STN (MEDLINE, BIOSIS, CAPLUS, EMBASE)	07/28/2009	KAB

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CONFIRMATION NO. 5532

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/722,018	06/18/2007	514	1649	SER-125

APPLICANTS

Giampiero De Luca, Conches, SWITZERLAND;

**** CONTINUING DATA ***************************

This application is a 371 of PCT/EP2005/056954 12/20/2005
which claims benefit of 60/638,669 12/22/2004

**** FOREIGN APPLICATIONS ***************************

EUROPEAN PATENT OFFICE (EPO) 04106909.7 12/22/2004

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12/10/2008

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☑ Yes ☐ No	☑ Yes ☐ No	/KIMBERLY BALLARD/ Examiner's Signature	☐ Initials	SWITZERLAND	0	20	1

ADDRESS

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GAINESVILLE, FL 32614
UNITED STATES

TITLE

CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

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EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	25	De-luca-gia$.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2009/07/28 19:37
L2	5077	cladribine leustatin (2-chlorodeoxyadenosine) (2-chloro-2'deoxyadenosine)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2009/07/28 19:38
L3	61883	multiple adj sclerosis	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2009/07/28 19:39
L4	1466	l2 and l3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2009/07/28 19:39
L5	1372	l4 and oral	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2009/07/28 19:39
L6	136	l2 same l3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2009/07/28 19:41

7/28/2009 7:43:17 PM
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June 18, 2007

INFORMATION DISCLOSURE
STATEMENT
Patent Application
Docket No. SER-125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Giampiero de Luca

Filed : June 18, 2007

For : Cladribine Regimen for Treating Multiple Sclerosis

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR §§1.97 AND 1.98

Sir:

In accordance with 37 C.F.R. § 1.56, the references listed on the attached form PTO/SB/08 are being brought to the attention of the Examiner for consideration in connection with the examination of the above-identified patent application. A copy of each cited reference is enclosed.

It is respectfully requested that the references cited on the attached form PTO/SB/08 be considered in the examination of the subject application and that their consideration be made of record.

Applicant respectfully asserts that the substantive provisions of 37 C.F.R. §§ 1.97 and 1.98 are met by the foregoing statement.

Respectfully submitted,

/FRANKCEISENSCHENK/

Frank C. Eisenschenk, Ph.D.
Patent Attorney
Registration No. 45,332
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

FCE/jps
Attachments: Form PTO/SB/08; copies of references cited therein.

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.B./

PTO/SB/08A (08-03)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 3

Complete if Known	
Application Number	
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Art Unit	
Examiner Name	K. Ballard
Attorney Docket Number	SER-125

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No. [1]	Document Number Number - Kind Code [2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	U1	US-			
	U2	US-			
	U3	US-			
	U4	US-			
	U5	US-			
	U6	US-			
	U7	US-			
	U8	US-			
	U9	US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No. [1]	Foreign Patent Document Country Code [3] - Number [4] - Kind Code [5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T [6]
	F1	WO 04/087101 A2	10/14/2004	Ivax Corporation	All	
	F2	EP 0 626 853 B1	04/26/200	The Scripps Research Institute	All	
	F3					
	F4					
	F5					
	F6					
	F7					

Examiner Signature		Date Considered	

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This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.B./

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 2 of 3

Complete if Known	
Application Number	
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Group Art Unit	
Examiner Name	K. Ballard
Attorney Docket Number	SER-125

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No. [1]	Include name of the author (in CAPITAL LETTERS), title of the article, (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	R1	BEUTLER, E. *et al.* "Marrow Suppression Produced by Repeated Doses of Cladribine", *Acta Haematol*, 1994, pp. 10-15, Vol. 91.	
	R2	BEUTLER, E. *et al.* "Treatment of Multiple Sclerosis and Other Autoimmune Diseases With Cladribine", *Seminars in Hematology*, January 1, 1996, pp. 45-52, Vol. 33, No. 1, Supplement 1.	
	R3	BEUTLER, E. *et al.* "The treatment of chronic progressive multiple sclerosis with cladribine", *Proc. Natl. Acad. Sci. USA*, February 1996, pp. 1716-1720, Vol. 93.	
	R4	ELLISON, G. *et al.* "Oral Cladribine for Multiple Sclerosis", *Neurology*, March 1997, P03.070, pp. A174-A175, Vol. 48, No. 3, XP008047069.	
	R5	GRIEB, P. *et al.* "Effect of Repeated Treatments with Cladribine (2-Chlorodeoxyadenosine) on Blood Counts in Multiple Sclerosis Patients", *Archivum Immunologiae et Therapiae Experimentalis*, 1995, pp. 323-327, Vol. 43, No. 5-6.	
	R6	KAZIMIERCZUK, Z. *et al.* "Synthesis of 2'-Deoxytubercidin, 2'-Deoxyadenosine, and Related 2'-Deoxynucleosides via a Novel Direct Stereospecific Sodium Salt Glycosylation Procedure", *J. Am. Chem. Soc.*, 1984, pp. 6379-6382, Vol. 106, No. 21.	
	R7	KURTZKE, J. "Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS)", *Neurology*, November 1983, pp. 1444-1452, Vol. 33.	
	R8	LANGTRY, H. *et al.* "Cladribine: A Review of its Use in Multiple Sclerosis", *Biodrugs*, May 1998, pp. 419-433, Vol. 9, No. 3.	
	R9	LASSMANN, H. *et al.* "Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy", *TRENDS in Molecular Medicine*, March 2001, pp. 115-121, Vol. 7, No. 3.	
	R10	LUBLIN, F. *et al.* "Defining the clinical course of multiple sclerosis: Results of an international survey", *Neurology*, April 1996, pp. 907-911, Vol. 46.	
	R11	LUCCHINETTI, C. *et al.* "Multiple sclerosis: recent developments in neuropathology, pathogenesis, magnetic resonance imaging studies and treatment", *Current Opinion in Neurology*, 2001, pp. 259-269, Vol. 14.	
	R12	MATTSON, D. "Update on the diagnosis of multiple sclerosis", *Expert Review of Neurotherapeutics*, May 2002, pp. 319-327, Vol. 2, No. 3.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

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U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 3 of 3

Complete if Known	
Application Number	
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Group Art Unit	
Examiner Name	K. Ballard
Attorney Docket Number	SER-125

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No. [1]	Include name of the author (in CAPITAL LETTERS), title of the article, (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	R13	MCDONALD, W. *et al.* "Recommended Diagnostic Criteria for Multiple Sclerosis: Guidlines from the International Panel on the Diagnosis of Multiple Sclerosis", *Annals of Neurology*, July 2001, pp. 121-127, Vol. 50, No. 1.	
	R14	MILLER, R. *et al.* "Therapeutic advances in ALS", *Neurology*, 1996, pp. S217, Vol. 47, Suppl. 4.	
	R15	NOSEWORTHY, J. *et al.* "Multiple Sclerosis", *The New England Journal of Medicine*, September 28, 2000, pp. 938-952, Vol. 343, No. 13.	
	R16	POSER, C. *et al.* "New Diagnostic Criteria for Multiple Sclerosis: Guidelines for Research Protocols", *Annals of Neurology*, March 1983, pp. 227-231, Vol. 13, No. 3.	
	R17	RICE, G. *et al.* "Cladribine and progressive MS: Clinical and MRI outcomes of a multicenter controlled trial", *Neurology*, March 2000, pp. 1145-1155, Vol. 54.	
	R18	ROMINE, J. *et al.* "A Double-Blind, Placebo-Controlled, Randomized Trial of Cladribine in Relapsing-Remitting Multiple Sclerosis", *Proceedings of the Association of American Physicians*, *January*/February 1999, pp. 35-44, Vol. 111, No. 1.	
	R19	SCHUMACHER, G. *et al.* "Problems of Experimental Trials of Therapy in Multiple Sclerosis: Report by the Panel on the Evaluation of Experimental Trials of Therapy in Multiple Sclerosis", *Annals New York Academy of Sciences*, March 31, 1965, pp. 552-568, Vol. 122.	
	R20	SELBY, R. *et al.* "Safety and Tolerability of Subcutaneous Cladribine Therapy in Progressive Multiple Sclerosis", *Can. J. Neurol. Sci.*, 1998, pp. 295-299, Vol. 25.	
	R21	SIPE, J. *et al.* "A neurologic rating scale (NRS) for use in multiple sclerosis", *Neurology*, October 1984, pp. 1368-1372, Vol. 34.	
	R22	STELMASIAK, Z. *et al.* "A pilot trial of cladribine (2-chlorodeoxyadenosine) in remitting-relapsing multiple sclerosis", *Med. Sci Monit.*, 1998, pp. 4-8, Vol. 4, No. 1.	
	R23		
	R24		

Examiner Signature	/Kimberly Ballard/	Date Considered	07/28/2009

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

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      (FILE 'HOME' ENTERED AT 19:58:17 ON 28 JUL 2009)

      FILE 'ZCAPLUS' ENTERED AT 19:58:37 ON 28 JUL 2009
                E CLADRIBINE/CT
                E E3+ALL

      FILE 'CAPLUS' ENTERED AT 19:59:29 ON 28 JUL 2009
                E US-2007722018/APPS

      FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 20:00:36 ON 28 JUL 2009
L1          7700 S CLADRIBINE OR 2-CHLORODEOXYADENOSINE OR 2-CHLORO-2-DEOXYADENO
L2        138027 S MULTIPLE(A)SCLEROSIS
L3           257 S L1(2P)L2
L4            88 S L3 AND ORAL?
L5            53 DUP REM L4 (35 DUPLICATES REMOVED)
L6           896 S DE-LUCA G/AU
L7             0 S L6 AND L1
L8            19 S L6 AND L2
L9            10 DUP REM L8 (9 DUPLICATES REMOVED)
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I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on December 18, 2009.

Frank C. Eisenschenk, Ph.D., Patent Attorney

AMENDMENT UNDER 37 C.F.R. § 1.111
Patent Application
Docket No. SER.125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Kimberly Ballard
Art Unit	:	1649
Applicant	:	Giampiero De Luca
Serial No.	:	11/722,018
Filed	:	June 18, 2007
Conf. No.	:	5532
For	:	Cladribine Regimen for Treating Multiple Sclerosis

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

AMENDMENT UNDER 37 C.F.R. § 1.111

Sir:

Applicants request that the period for response be extended two months through and including January 4, 2010, the fees for which have been paid at the time this Amendment was filed.

In response to the Office Action dated August 3, 2009, please amend the above-identified patent application as follows:

In the Claims

1-17 (canceled).

18 (currently amended). A method of treating multiple sclerosis comprising the oral administration of a formulation comprising cladribine, wherein the formulation is to be orally administered following the sequential steps below:

(i) an induction period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period of between about 8 and about 10 months wherein no cladribine formulation is administered;

(iii) a maintenance period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the maintenance period is lower than the total dose of cladribine reached at the end of the induction period (i); and

(iv) a cladribine-free period wherein no cladribine formulation is administered.

19 (currently amended). The method according to claim 18, wherein the induction period lasts up to about 4 months~~, or up to 3 months, or up to 2 months~~.

20 (currently amended). The method according to claim 19, wherein the induction period lasts ~~up to~~ about 2 months.

21 (currently amended). The method according to ~~claim 18~~ claim 19, wherein the induction period lasts ~~up to 2 months~~ about 3 months.

22 (currently amended). The method according to ~~claim 18~~claim 19, wherein the induction period lasts ~~up to~~ about 4 months.

23 (currently amended). The method according to claim 19, wherein the induction period lasts up to about 3 months~~4 months~~.

24 (currently amended). The method according to claim 18, wherein the total dose of cladribine reached at the end of the induction period is about 1.7 mg/kg.

25 (currently amended). The method according to claim 18, wherein the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg.

26 (canceled).

27 (currently amended). The method according to claim 18, wherein the cladribine-free (iv) period lasts ~~up to~~ about 10 months.

28 (currently amended). The method according to claim 18, wherein the maintenance period lasts ~~up to~~ about 4 months~~, or up to 3 months or up to 2 months~~.

29 (currently amended). The method according to claim 18, wherein the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

30 (currently amended). The method according to claim 18, wherein the formulation is to be orally administered following the sequential steps below:

(i) an induction period wherein said cladribine formulation is orally administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period of between about 8 and about 10 months wherein no cladribine formulation is administered;

(iii) a maintenance period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the maintenance period is lower than the total dose of cladribine reached at the end of the induction period (i); and

(iv) a cladribine-free period wherein no cladribine formulation is administered;

wherein the induction period lasts up to 4 months~~, or up to 3 months or up to 2 months~~; the cladribine-free period (ii) lasts up to 10 months~~, or up to 8 months or up to 10 months~~; the maintenance period (iii) lasts up to 2 months; the cladribine-free period (iv) lasts up to 10 months; the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated ~~performed~~ one, two or three times.

31 (currently amended). The method according to claim 30, wherein the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

32 (previously presented). The method according to claim 30, wherein the formulation is to be orally administered at a daily dose of 3 to 30 mg cladribine.

33 (currently amended). The method according to claim 32, wherein the ~~pharmaceutical~~ formulation is to be orally administered at a daily dose of 10 mg cladribine.

34 (currently amended). The method according to claim 18, wherein the ~~pharmaceutical~~ formulation is orally administered 1 to 7 days per month during the induction period.

35 (previously presented). The method according to claim 18, wherein the steps (iii) to (iv) are repeated at least one or two times.

36 (previously presented). The method according to claim 18, wherein said cladribine formulation is to be administered in combination with interferon-beta.

37 (previously presented). The method according to claim 30, wherein said cladribine formulation is to be administered in combination with interferon-beta.

38 (new). A method of treating multiple sclerosis comprising the oral administration of a formulation comprising cladribine following the sequential steps below:

(i) an induction period lasting from about 2 months to about 4 months wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period lasting from about 8 months to about 10 months, wherein no cladribine is administered;

(iii) a maintenance period lasting from about 2 months to about 4 months, wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the maintenance period is lower than the total dose of cladribine reached at the end of the induction period (i);

(iv) a cladribine-free period wherein no cladribine is administered.

39 (new). The method according to claim 38, wherein the induction period lasts about 4 months.

40 (new). The method according to claim 38, wherein the induction period lasts about 2 months.

41 (new). The method according to claim 38, wherein the total dose of cladribine reached at the end of the induction period is about 1.7 mg/kg.

42 (new). The method according to claim 38, where the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg.

43 (new). The method according to claim 38, wherein the cladribine-free period (ii) lasts about 10 months.

44 (new). The method according to claim 38, wherein the cladribine-free (iv) period lasts 10 months.

45 (new). The method according to claim 38, wherein the maintenance period lasts about 2 months.

46 (new). The method according to claim 38, wherein the formulation is orally administered following the sequential steps below:

(i) an induction period wherein said formulation is administered orally and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period wherein no cladribine is administered;

(iii) a maintenance period wherein said formulation is administered orally and wherein the total dose of cladribine reached at the end of the maintenance period is lower than the total dose of cladribine reached at the end of the induction period (i);

(iv) a cladribine-free period wherein no cladribine is administered.

wherein the maintenance period (iii) lasts about 2 months; the cladribine-free period (iv) lasts about 10 months; the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeatedly performed one, two or three times.

47 (new). The method according to claim 38, wherein the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

48 (new). The method according to claim 38, wherein the formulation is orally administered at a daily dose of 3 to 30 mg cladribine.

49 (new). The method according to claim 38, wherein the formulation is orally administered at a daily dose of 10 mg cladribine.

50 (new). The method according to claim 38, wherein the formulation is orally administered 1 to 7 days per month during the induction period.

51 (new). The method according to claim 38, wherein the steps (iii) to (iv) are repeated at least one time.

52 (new). The method according to claim 38, wherein the steps (iii) to (iv) are repeated at least two times.

53 (new). The method according to claim 38, wherein the formulation is administered in combination with interferon-beta.

54 (new). A method of treating multiple sclerosis comprising the oral administration of a formulation comprising cladribine following the sequential steps below:

(i) an induction period lasting from about 2 months to about 4 months wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period lasting from about 8 months to about 10 months, wherein no cladribine is administered;

(iii) a maintenance period lasting from about 2 months to about 4 months, wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg;

(iv) a cladribine-free period wherein no cladribine is administered.

55 (new). The method according to claim 54, wherein the induction period lasts about 4 months.

56 (new). The method according to claim 54, wherein the induction period lasts about 2 months.

57 (new). The method according to claim 54, wherein the total dose of cladribine reached at the end of the induction period is about 1.7 mg/kg.

58 (new). The method according to claim 54, where the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg.

59 (new). The method according to claim 54, wherein the cladribine-free period (ii) lasts about 10 months.

60 (new). The method according to claim 54, wherein the cladribine-free (iv) period lasts 10 months.

61 (new). The method according to claim 54, wherein the maintenance period lasts about 2 months.

62 (new). The method according to claim 54, wherein the formulation is orally administered at a daily dose of 3 to 30 mg cladribine.

63 (new). The method according to claim 54, wherein the formulation is orally administered at a daily dose of 10 mg cladribine.

64 (new). The method according to claim 54, wherein the formulation is orally administered 1 to 7 days per month during the induction period.

65 (new). The method according to claim 54, wherein the steps (iii) to (iv) are repeated at least one or two times.

66 (new). The method according to claim 54, wherein the formulation is administered in combination with interferon-beta.

Remarks

Claims 18-37 are pending in the subject application. By this Amendment, Applicant has canceled claim 26, amended claims 18-25, 27-31, 33 and 34 and added new claims 38-66. Support for the amendments and new claims can be found throughout the subject specification and in the claims as originally filed (see, for example, page 7, line 15 through page 8, line 3; and pages 13-18 of the as-filed application). Entry and consideration of the amendments and new claims presented herein is respectfully requested. Accordingly, claims 18-25 and 27-66 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

The courtesy of an interview in this matter is requested at the time the Examiner considers this response.

Claim 30 is objected to because of informalities. By this Amendment, the repeated phrase "up to 10 months" has been deleted. Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

Claims 18-37 are rejected under 35 U.S.C. § 103(a) as obvious over Bodor *et al.* (WO 2004/087101) and Beutler (U.S. Patent No. 5,506,213) in view of Bloom (U.S. Patent No. 4,964,848). The Office Action indicates that the Bodor *et al.* and Beutler references teach the use of cladribine for the treatment of multiple sclerosis. The Office Action argues, at pages 3-8:

> Specifically, Bodor discloses treatment of multiple sclerosis comprising administering to a patient in need thereof a therapeutically effect amount of a composition comprising cladribine, wherein the composition is formulated for oral administration. In particular, Bodor *et al.* teaches that for the treatment of multiple sclerosis, 10 mg of cladribine in solid dosage form is to be administered orally once per day for a period of five to seven days in the first month, repeated for another period of five to seven days in the second month, followed by ten months of no treatment. Alternatively, the patient may be treated with 10 mg of cladribine in the dosage form once per day for a period of five to seven days per month for a total of six months, followed by eighteen months of no treatment. Further, Bodor *et al.* discloses that one of skill in the art will appreciate that the therapeutically effective amount of cladribine administered may be lowered or increased by fine tuning and/or by administering cladribine with another active ingredient.
>
> Hence, the teachings of Bodor *et al.* address the following recited limitations of the instant claims: an induction period lasting up to 4 months, or up to 3 months, or up to 2 months; the cladribine-free period lasts up to 10 months; the maintenance period lasts up to 4 months, or up to 3 months, or up to 2 months; the formulation is

to be orally administered at a daily dose of 3 to 30 mg cladribine or at a daily dose of 10 mg cladribine; and the pharmaceutical formulation is administered 1 to 7 days per month during the induction period.

Finally, regarding claims 36 and 37, Bodor *et al.* disclose administration of cladribine in conjunction with the administration of one or more additional active ingredients. For example, in the treatment of multiple sclerosis, Bodor *et al.* teach co-administration of interferon beta.

With regard to the purported teachings of Bodor *et al.*, Applicant notes that the reference is silent with respect to the administration of cladribine therapy after the cladribine-free period of between about 8 and 10 months. Thus, Bodor *et al.* fails to teach a cladribine-free period of between about 8 and 10 months followed by a "maintenance period" during which a cladribine formulation is administered such that the total dose administered in the "maintenance period" is lower than the total dose first administered to the patient which is then followed by another cladribine-free period.

The Office Action further argues:

In accord with the teachings of Bodor *et al.*, Beutler discloses a method of treating multiple sclerosis by administration of 2-chloro-2'-deoxyadenosine (a.k.a. cladribine), wherein oral administration is a preferred mode of administration. Beutler teaches that for oral administration, a therapeutically effective daily dose can range from about 0.04 mg/kg to about 1.0 mg/kg/day. Typical administration lasts for a time period of about 5 to about 14 days, with a 7-day time course being usual. Courses (cycles) of administration can also be repeated at monthly intervals. Oral unit dosages can be administered at intervals of one to several days to provide the therapeutically effective dose. Thus, for an average adult human weighing 150 lbs (68 kg), according to the claimed invention, if the total dose reached at the end of the induction period is 3.5 mg/kg and the total dose reached at the end of the maintenance period is 1.7 mg/kg, for example, this would amount to 238 mg and 115.6 mg cladribine, respectively. According to Beutler, a 68 kg adult receiving oral cladribine therapy for 7 days, for example, could receive a total dose of between 19.04 and 476 mg cladribine, which encompasses the instantly recited doses of claims 18, 24,25, and 29-31. Thus, Beutler notes, *in vivo* administration of the above dosages over a time period of about 5 to about 14 days or at weekly or day intervals provides an amount sufficient to kill at least 50 percent of the originally present monocytes (which acts to down-regulate the autoimmune aspect of MS). Beutler further teaches that the daily administration course can be repeated periodically over a period of several months, e.g. about three to about nine months. In usual practice, this means that treatments are administered over a period of about 5-7 days and are repeated at about 3 to about 4 week intervals for several months, e.g. about 3 to about 9 months. Therefore, for a 7 day administration period (*i.e.*, the induction period), the

> total dose of cladribine reached at the end of the 7 days would be in the range of 0.28 to 7.0 mg/kg, which would encompass the instantly recited range of 1.7 to 3.5 mg/kg. And in addition to addressing presently claimed limitations regarding the frequency of the administration cycles, Beutler's disclosure also addresses instantly recited limitations of claims 30 and 35, which state that the maintenance (iii) step and cladribine-free period (iv) step are repeated at least one, two or three times.

In this regard, Applicant notes that Beutler fails to teach any period of time that corresponds to a "cladribine-free period" as recited in the instant claims. Rather, Beutler teaches methods of treating multiple sclerosis comprising the administration of adenosine compounds, such as cladribine, that is repeated periodically, such as weekly or monthly over a period of several months to about one year (column 9, line 61 through column 10, line 7). In column 11 (at lines 1-15), Beutler discusses the typical cladribine administration cycles. In this passage, the typical administration period is about 5 to about 14 days (with a seven day time course typical) followed by a period during which cladribine is not administered. Where the treatment cycle is monthly, the "cladribine-free" period ranges from about 23-26 days (depending on the month for a 5 day administration period) to as little as 14-17 days (depending on the month for a 14 day administration period). Applicant submits that no passage of Beutler discloses or contemplates cladribine-free periods that range from about 8 to about 10 months.

The Office Action further argues:

> Taken together, the combined teachings of Bodor *et al.* and Beutler provide for method of treating multiple sclerosis comprising oral administration of cladribine, wherein a typical treatment course comprises the daily administration of cladribine for 1-7 days (i.e., induction phase) followed by a cladribine-free period and then another course of daily cladribine for 1-7 days in a subsequent treatment such as the second month i.e., maintenance phase), followed by up to 10 months of no treatment, wherein a weekly treatment course can be repeated periodically for several months. However, neither of the above references teach that the total dose of cladribine reached at the end of the maintenance phase is lower than the total dose reached at the end of the induction phase.

Applicant respectfully submits that the combined teachings of Bodor *et al.* and Beutler do not give rise to the method of treating multiple sclerosis as asserted in the Office Action. For example, there is no teaching in Bodor *et al.* regarding repeated treatment cycles comprising the administration of

cladribine after the 10 month cladribine free period and the Office Action points to no such teachings. While Beutler discloses multiple treatment "cycles", the teachings of that reference relate to the repeated weekly or monthly administration of cladribine over a period of 5 to 14 days within each "cycle".

The Office Action also notes other deficiencies in the teachings of Bodor *et al.* and Beutler. Notably, that "neither of the above references teach that the total dose of cladribine reached at the end of the maintenance phase is lower than the total dose reached at the end of the induction phase" (Office Action at the paragraph bridging pages 6-7). In an effort to cure this deficiency, the Office Action argues (citing to the teachings of Bloom at page 7 of the Office Action):

> Bloom discusses the treatment of multiple sclerosis and other autoimmune diseases, wherein effective treatment requires an intense induction phase lasting from about five to seven weeks during which time lymphocytes are continuously depleted from circulation to a level of less than 500 cells/μl, followed by a maintenance phase in a lower intensity treatment regimen is employed to hold the overall blood lymphocyte count to less than 500 cell/μl. While the initial induction phase of treatment is accomplished here by lymphocytapheresis and the maintenance phase is accomplished with the use of immunosuppressive or immunomodulatory agents other than cladribine, the principle of Bloom's teachings remains the same: effective treatment of multiple sclerosis requires an intense induction phase with substantial depletion of blood lymphocytes followed by a more moderate maintenance phase to hold the cell numbers down. Even with respect to the immunomodulatory agents employed in the maintenance phase, Bloom teaches that treatment dosages are lowered with each successive use of the drug (see, for example, column 4, lines 39-49 regarding the use of azathioprine (AZA) and prednisone).

Applicant respectfully disputes the alleged teachings of Bloom proffered in the Office Action. For example, the Office Action argues (at page 7) that Bloom teaches an induction period followed by a maintenance phase where "a lower intensity treatment regimen is employed to hold the overall blood lymphocyte count to less than 500 cell/μl. While the initial induction phase of treatment is accomplished here by lymphocytapheresis and the maintenance phase is accomplished with the use of immunosuppressive or immunomodulatory agents other than cladribine, the principle of Bloom's teachings remains the same: effective treatment of multiple sclerosis requires an intense induction phase with substantial depletion of blood lymphocytes followed by a more moderate maintenance phase to hold the cell numbers down". Such is not, in fact, the case.

As noted at column 4, lines 18-52 of Bloom, both lymphocytapheresis and a chemotherapeutic protocol are administered to an individual to hold lymphocyte numbers down to the desired levels (less than 500 cells/μL). It is unclear how this combined therapeutic regimen, including a combination of immunosuppressive drugs (AZA and prednisone) added to lymphocytapheresis can be construed as "a more moderate maintenance phase" or a "lower intensity treatment regimen". If anything, this combination protocol taught in Bloom would have been considered a more intense treatment regimen since both lymphocytapheresis and immunosuppression via AZA and prednisone were being employed on the subject.

Applicant further notes that the alleged rationale for combining Bloom with either Bodor *et al.* and/or Beutler, namely, "the principle of Bloom's teachings remains the same: effective treatment of multiple sclerosis requires an intense induction phase with substantial depletion of blood lymphocytes followed by a more moderate maintenance phase to hold the cell numbers down" would not be logical in view of the teachings of Beutler, who indicates that the level of circulating monocytes returns to pretreatment levels about two weeks after treatment with cladribine is stopped (see column 11, lines 22-24). As such, the teachings of Beutler suggests that it would not have been possible to "hold the cell numbers down" since the levels of circulating monocytes return to pretreatment levels about two weeks after cladribine treatment is stopped. Thus, Applicant submits that one skilled in the art would not have had a reasonable expectation of success in holding cell numbers down in view of the express teachings of Beutler where a treatment protocol such as that claimed in this application was followed. Indeed, the teachings of Beutler indicate to one skilled in the art that a cladribine-free period that exceeded more than two weeks was undesirable since total monocyte numbers would rebound to pretreatment levels about two weeks after cladribine administration ceased.

Turning to the argument that "[e]ven with respect to the immunomodulatory agents employed in the maintenance phase, Bloom teaches that treatment dosages are lowered with each successive use of the drug (see, for example, column 4, lines 39-49 regarding the use of azathioprine (AZA) and prednisone)". Applicant submits that this is an overstatement of the teachings of the reference. While it is true that the dosage of AZA and prednisone are lowered during the course of administration, the AZA dose is lowered from 5 mg/kg/day to 2.5 mg/kg/day over the span of five

(5) days where it is held constant for the course of treatment (at 2.5 mg/kg). Likewise, prednisone is reduced from 60 mg/kg/day to 15 mg/kg/day over the span of 10 weeks and held constant at that dosage (15 mg/kg/day) for at least one year. Thus, it is clear that Bloom does not teach lowering treatment dosages with each successive use of the drug, rather Bloom teaches the tapering the dosage of each drug to maintenance dosages that are maintained throughout the treatment protocol.

The Office Action further argues that:

> Thus, as evidenced by the prior art, the skilled artisan would have known that effective treatment of multiple sclerosis involves the removal of the majority (e.g., up to 90%; see Beutler, column 11) of activated lymphocytes from the patient in an initial phase of the treatment, followed by a maintenance phase in which the numbers of lymphocytes are maintained at a reduced level. The artisan would have also been aware that cladribine is useful for the treatment of multiple sclerosis, as cladribine is an immunosuppressive agent that functions by killing activated lymphocytes (and in particular, monocytes, which are presumably activated against self-antigens). Particularly in view of the chronic nature of multiple sclerosis, in order to reduce the severity or duration of future relapses (or to prevent them altogether) the artisan would have been aware that in order for long-term treatment to be successful, it must be sustainable and well-tolerated by the patient. Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to treat multiple sclerosis with oral cladribine according to a cyclic treatment regimen, wherein treatment involves an induction phase and a maintenance phase, and wherein the total dosage administered in the maintenance phase is less than the total dosage administered in the induction phase. Additionally, even though cladribine therapy is generally well-tolerated with a low incidence of adverse effects (see, for example, Examples 1 and 2 in the Beutler patent), in order to reduce overall treatment costs associated with cladribine therapy and further lessen the risk of negative side effects, the artisan would have been motivated to use a lower dose in the maintenance phase that is still sufficient to sustain a therapeutically-effective immunosuppressive state.

As noted above, it would not have been obvious to one of ordinary skill in the art at the time the invention was made that effective treatment of multiple sclerosis involved the removal of the majority of activated lymphocytes from the patient in an initial phase of the treatment, followed by a maintenance phase in which the numbers of lymphocytes are maintained at a reduced level. Indeed, the express teachings of Beutler indicate that total monocyte numbers rebound to pretreatment levels about two weeks after cladribine administration is stopped (column 11, lines 20-24). Thus, one skilled in the art would not have had a reasonable expectation of success in maintaining lower

lymphocyte numbers in view of the express teachings of Beutler where a treatment protocol such as that claimed in this application was administered.

One of the final arguments set forth in the Office Action argues:

> Regardless, each of the recited doses, treatment durations, and frequencies are clearly result effective parameters that a person of ordinary skill in the art would routinely optimize (see M.P.E.P § 2144.05). Optimization of parameters is a routine practice that would be obvious for a person of ordinary skill in the art to employ. Indeed, both Bodor *et al.* and Beutler assert that it is within the level and skill of the artisan to fine-tune cladribine dosages and treatment protocols in order to achieve a desired therapeutic effect. Thus, absent some demonstration of unexpected results from the claimed parameters, this optimization would have been obvious at the time of applicant's invention. Furthermore, as was noted by the United States Supreme Court, if a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one method (e.g., Bloom's method of high dose, no dose, low dose), and a person of ordinary skill would recognize that it would improve similar methods (e.g., Bodor *et al.* or Beutler) in the same way, using the technique is obvious unless its actual application is beyond his or her skill. *KSR,* 127 S. Ct. at 1740. "When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product is not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show it was obvious under 35 U.S.C. 103." *KSR Int'I Co. v. Teleflex Inc.,* 127 S.Ct. 1727, 1742, 82USPQ2d 1385, 1396 (2007).

First, Applicant notes that the Court of Customs and Patent Appeals has held that a particular parameter must first be recognized as a result-effective variable, *i.e.*, a variable which achieves a recognized result, before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation (*see In re Antonie*, 559 F.2d 618, 620 (C.C.P.A. 1977)). Applicant respectfully submits that the Office Action fails to provide any evidence that a dosing regimen, such as that claimed herein, was recognized to be a "result effective variable" that achieved a recognized result. While the Office Action argues that "both Bodor *et al.* and Beutler assert that it is within the level and skill of the artisan to fine-tune cladribine dosages and treatment protocols in order to achieve a desired therapeutic effect", this is far from a teaching that variations in the timing of cladribine administration would have a desired therapeutic effect or could be optimized. At best,

the teachings in each of those references might suggest or teach that the amount of cladribine administered to an individual patient should be "fine-tuned" to achieve a desired therapeutic effect. Applicants further submit that there is no evidence of record that suggests that the claimed dosing regimen could be derived by "fine-tuning" of dosing teachings of Bodor *et al.* and/or Beutler.

Applicant further submits that reliance on Bloom for the purported teaching that "if a technique has been used to improve one method (*e.g.*, Bloom's method of high dose, no dose, low dose), and a person of ordinary skill would recognize that it would improve similar methods (*e.g.*, Bodor *et al.* or Beutler) in the same way, using the technique is obvious unless its actual application is beyond his or her skill." Applicant submits that, as discussed above, Bloom fails to teach a "method of high dose, no dose, low dose" treatment. As noted above, Bloom's maintenance phase, if anything, is a higher intensity therapeutic regimen that utilized immunosuppression via **AZA and prednisone in combination with lymphocytapheresis** to maintain blood lymphocyte levels at the desired levels of less than 500 cells/μL. Furthermore, one skilled in the art would not have had a reasonable expectation of success in keeping blood lymphocyte levels at fewer than 500 cells/μL since Beutler expressly teaches circulating monocyte levels rebound to pretreatment levels about two weeks after cladribine treatment is stopped (column 11, lines 21-24). Thus, one skilled in the art would not expect to be able to maintain monocyte levels at the required levels where a cladribine-free period exceeded about two weeks.

As the Patent Office is aware, all the claim limitations must be taught or suggested by the prior art in order to establish a *prima facie* case of obviousness. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (C.C.P.A. 1974). Furthermore, "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 41 (2007). The Examiner is required to explicitly demonstrate that "there was an apparent reason to combine the known elements in the fashion claimed" by the applicant, "other than the hindsight gleaned from the invention itself." *Interconnect Planning Corp. v. Feil,* 774 F.2d 1132, 1143 (Fed. Cir. 1985), *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 41 (2007). In addition, a reasonable expectation of success is required to establish a *prima facie* case of obviousness (*see* M.P.E.P. § 2143.02)

Applicant also respectfully submits that Bloom is non-analogous art. As the Patent Office is aware, the Patent Office's reviewing court has found that the classification of references is some evidence of "nonanalogy" or "analogy" with respect to the use of a reference in an obviousness rejection. In this regard, Applicant notes that Bloom is not classified in the same area as the instant invention. For example, the instant application is classified in Class 424, subclass 085.600 (Drug, Bio-Affecting And Body Treating Compositions) whereas the invention of Bloom is classified in the surgery (Class 604). Applicant submits that one skilled in the art would not have looked to patents issued into the surgery class for method of treating multiple sclerosis using drugs, such as cladribine. Additionally, Applicant also submits that one skilled in the art, seeking to treat multiple sclerosis using a drug regimen, would not have looked to the surgical arts and methods that extracted blood from a subject and then replaced or returned that blood to the body of the subject being treated (the inventions found in Class 604, subclasses 4-6; see attached classification index).

Applicant respectfully asserts that the claimed invention is not obvious over the cited references and that a *prima facie* case of obviousness has not been established because each of the limitations of the claimed invention has not been taught or suggested by the combination of references. Additionally, as discussed above, there is no apparent reason to combine the cited references absent the teachings of Applicant's specification and one of ordinary skill in the art would not have had a reasonable expectation of success in arriving at the claimed treatment protocol in view of the cited combination of references (particularly in view of the express teachings of Beutler). Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

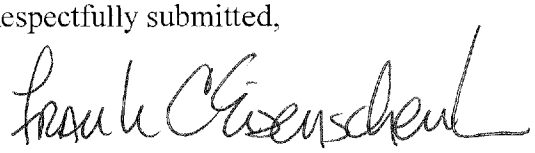
It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicant's agreement with or acquiescence in the Examiner's position. Applicant expressly reserves the right to pursue the invention(s) disclosed in the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicant believes that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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FCE/sl
Attachment: Classification index

This Class 604 is considered to be an integral part of Class 128 (see the Class 128 schedule for the position of this Class in schedule hierarchy). This Class retains all pertinent definitions and class lines of Class 128.

890.1 **CONTROLLED RELEASE THERAPEUTIC DEVICE OR SYSTEM**

891.1 .Implanted dynamic device or system

892.1 .Osmotic or diffusion pumped device or system

1 **SWAB INCLUDING HANDLE (E.G., STICK, ETC.) WITH ABSORBENT MATERIAL AT END THEREOF**

2 .Body treating material fed to absorbent material

3 ..Means broken, cut, pierced, or torn to permit flow of material

4.01 **BLOOD DRAWN AND REPLACED OR TREATED AND RETURNED TO BODY**

5.01 .Constituent removed from blood and remainder returned to body

5.02 ..Pathogenic component removed

5.03 ..Lipidic material removed

5.04 ..Toxic material removed

6.01 ..Component of blood removed (i.e., pheresis)

6.02 ...Erythrocyte

6.03 ...Leukocyte

6.04 ...Plasma

6.05 ...Single needle

6.06 ...Arterial and venous needles

6.07 ..Anticoagulant added

6.08 ..Infrared, visible light, ultraviolet, x-ray or electrical energy applied into blood

6.09 ..Filter means

6.1 ..Valve means

6.11 ..Pumping means

6.12 ...Injector or aspirator syringe supported only by person during use

6.13 ..Heating or cooling means

6.14 ..Oxygenating means

6.15 .Blood collection container

6.16 .Body inserted tubular conduit structure

7 **BLOOD TRANSFERRED BETWEEN DIFFERENT BODIES ALONG CONTINUOUS FLOW PATH (E.G., TRANSFUSION, ETC.)**

8 **DEVICES TRANSFERRING FLUIDS FROM WITHIN ONE AREA OF BODY TO ANOTHER (E.G., SHUNTS, ETC.)**

9 .With flow control means (e.g., check valves, hydrocephalus pumps, etc.)

10 ..With antisiphon means

11 **MEANS FOR INSERTING FIBROUS OR FORAMINOUS RESIDENT PACKING, RECEPTOR, OR MEDICAMENT CARRIER INTO BODY ORIFICE**

12 .With lubricating means

13 .With means for ejecting continuous length insert (e.g., gauze packing)

14 .Distal portion of inserting means deformed, expanded, or ruptured to permit passage of insert therefrom

15 .With slidable ejector (e.g., plunger or ram, etc.) inside tubular inserting means

16 ..Ejector moved into operating position from stored location inside or alongside inserting means

17 ...Ejector pivoted or swung into operating position

18 ..Tubular inserting means releasably interlocked with ejector

19 **MEANS FOR INTRODUCING OR REMOVING MATERIAL FROM BODY FOR THERAPEUTIC PURPOSES (E.G., MEDICATING, IRRIGATING, ASPIRATING, ETC.)**

20 .Infrared, visible light, ultraviolet, X-ray or electrical energy applied to body (e.g., iontophoresis, etc.)

21 ..With tubular injection means inserted into body

22 .With means for cutting, scarifying, or vibrating (e.g., ultrasonic, etc.) tissue

23 .Gas application

Class 600 is an integral part of this Class (Class 128), as shown by the position of this box, and follows the schedule hierarchy of this Class, retaining all pertinent definitions and Class lines of this class.

Class 601 is an integral part of this Class (Class 128), as shown by the position of this box, and follows the schedule hierarchy of this Class, retaining all pertinent definitions and Class lines of this class.

Class 602 is an integral part of this Class (Class 128), as shown by the position of this box, and follows the schedule hierarchy of this Class, retaining all pertinent definitions and Class lines of this class.

95.1 **TRUSS**
96.1 .Abdominal
97.1 .Head
98.1 .Perineal
99.1 .Support
100.1 ..Belt wholly flexible
101.1 ...Elastic in part
102.1 ..Belt and frame
103.1 ..Frame hinged
104.1 ..Frame wholly metallic
105.1 ..Frame with auxiliary straps
106.1 ..Pad carrier
107.1 ...Detachable
108.1 ...Pivoted
109.1 ...Resilient
110.1 ....Clamped
111.1 ...Resilient
112.1 .Pad
113.1 ..Composition
114.1 ..Medicating
115.1 ..Rigid
116.1 ..Adjustable center
117.1 ..Resilient
118.1 ...Inflated
119.1 ...Spring
120.1 ...Stuffed
121.1 ..Connections
122.1 ...Ball and socket
123.1 ...Hinged
124.1 ...Clamped
125.1 ...Resilient
126.1 ....Spring only
830 **FEMALE REPRODUCTORY TRACT SHIELDS, SUPPORTS, OR BIRTH CONTROL DEVICES (E.G., PESSARIES, CONTRACEPTIVE DEVICES)**
831 .Fallopian occluders
832 .With contraceptive, spermacidal, or antifertility agent
833 ..Intrauterine
834 .Pessaries
835 ..External supporters
836 ..Inflatable
837 ..Diaphragm
838 ...Inserters and removers
839 ..Intrauterine
840 ...Inserters and removers
841 ...With cervical cap
842 **MALE REPRODUCTORY TRACT SHIELDS OR BIRTH CONTROL DEVICES (E.G., PROPHYLACTICS, VAS DEFERENS VALVES, ETC.)**
843 .Vas occluders (implants, etc.)
844 .Condoms
845 **BODY RESTS, SUPPORTS OR POSITIONERS FOR THERAPEUTIC PURPOSE (E.G., SEXUAL, POSTURAL, HEAD, ETC.)**
846 **BODY PROTECTING OR RESTRAINING DEVICES FOR PATIENTS OR INFANTS (E.G., SHIELDS, IMMOBILIZERS)**
847 .With fluid supply
848 .Antisnoring device
849 .Drapes
850 ..Incision or cavity inserted
851 ..With handle or applicator means
852 ..With surgical implement retaining means
853 ..Fenestrated
854 ...With cover (flap)
855 ..Folded or stacked
856 ..Tubular
857 .Head or face protector (e.g., lips, ears, etc.)
858 ..Eye or nose protectors
859 ..Oral cavity protectors
860 ...Tongue
861 ...Teeth protectors (e.g., mouthpieces)
862 ....Thermoplastic or thermosetting type
863 ..Breath or contaminated air deflectors

204.13 ..Treating agent evaporated from extended surface absorbent (e.g., sponge, fibrous wick, screen, etc.)
204.14 ..Respiratory gas passed over surface of liquid treating agent in reservoir
204.15 .Means for cooling respiratory gas or respiration device
204.16 ..Substance removed from respiratory gas by cooling
204.17 .Means for heating respiratory gas or respiration device
204.18 .Means for supplying respiratory gas under positive pressure
204.19 ..Permanent magnet included in gas flow control means
204.21 ..Electric control means
204.22 ...Means for sensing partial pressure, or amount, of component in gas mixture
204.23 ...Means for sensing condition of user's body
204.24 ..Fluidic control device utilizing Coanda effect or jet impingement to alter fluid flow
204.25 ..Gas stream passed through jet nozzle or venturi passage
204.26 ..Gas supply means responsive to breathing
204.27 ...Snap action toggle included in control mechanism
204.28 ...Bag or bellows included in control mechanism
204.29 ..Control means responsive to variation in ambient pressure
205.11 ..Means for varying air/oxygen ratio
205.12 ..Means for removing substance from respiratory gas
205.13 ..Respiratory gas supplied from expandable bag, bellows, or squeeze bulb
205.14 ...Means for adjusting gas volume delivered to user from bag, bellows, or bulb during inflation-deflation cycle
205.15 ...Held in pressurizable housing
205.16 ...Biased to contracted or expanded position by mechanical means (e.g., weight, spring, etc.)
205.17 ...Rebreathing bag or bellows
205.18 ..Gas supplied by piston pump
205.19 ..Suction means for assisting exhalation
205.21 ..Means broken or pierced to supply gas
205.22 ..Gas container supported on body
205.23 ..Indicator structure
205.24 ..Valve, or valve control, structure
205.25 ..Face mask covering a breathing passage
205.26 ..Atmosphere enclosure (e.g., oxygen tent, hyperbaric chamber for pressurizing whole body, etc.)
205.27 .Means for removing substance from respiratory gas
205.28 ..Carbon dioxide
205.29 ..Particulate filtering
206.11 ...Including means inserted in nasal passage
206.12 ...Face mask covering a breathing passage
206.13 ....Mask attached to ear
206.14 ....Mask adhesively attached to face
206.15 ....With gas flow control valve
206.16 ....With frame, shaping means, reinforcement, or filter formed of wire
206.17 ....With separate filter encircling element, or housing, securing filter on mask
206.18 ....Covering nose only
206.19 ....Body of mask, other than viewing means, formed of porous filter material (e.g., surgical mask formed entirely of cloth, etc.)
206.21 .Face mask covering a breathing passage
206.22 ..Means for handling liquid (e.g., saliva, breath condensation, etc.) accumulated in mask
206.23 ..Mask/eyepiece sealing structure
206.24 ..Mask/face sealing structure
206.25 ...Adhesive
206.26 ...Closed air-filled passage adjacent mask edge (e.g., tubular bead, etc.)
206.27 ..Means holding mask readily accessible for use
206.28 ..Covering nose and mouth

206.29 ...Including means inserted in mouth
207.11 ...Structure of means securing mask to head
207.12 ...Valve for controlling gas flow
207.13 ..Covering nose only
207.14 .Respiratory gas supply means enters mouth or tracheotomy incision
207.15 ..Breathing passage occluder
207.16 ..Valve for controlling gas flow
207.17 ..Holding strap extending circumferentially of head or neck
207.18 .Respiratory gas supply means enters nasal passage
207.29 **DEVICE FOR CREATING A TRACHEOTOMY INCISION**

Class 604 is an integral part of this Class (Class 128), as shown by the position of this box, and follows the schedule hierarchy of this Class, retaining all pertinent definitions and Class lines of this class.

Class 606 is an integral part of this Class (Class 128), as shown by the position of this box, and follows the schedule hierarchy of this Class, retaining all pertinent definitions and Class lines of this class.

Class 607 is an integral part of this Class (Class 128), as shown by the position of this box, and follows the schedule hierarchy of this Class, retaining all pertinent definitions and Class lines of this class.

897 **MISCELLANEOUS**
898 .Methods
899 .Devices placed entirely within body and means used therewith (e.g., magnetic implant locator)

CROSS-REFERENCE ART COLLECTIONS

900 **BLOOD PRESSURE RECORDER**
901 **SUPPRESSION OF NOISE IN ELECTRIC SIGNAL**
902 **BIOLOGICAL SIGNAL AMPLIFIER**
903 **RADIO TELEMETRY**
904 **TELEPHONE TELEMETRY**
905 **FEEDBACK TO PATIENT OF BIOLOGICAL SIGNAL OTHER THAN BRAIN ELECTRIC SIGNAL**
906 **MULTIPHASIC DIAGNOSTIC CLINIC**
907 **ACUPUNCTURE**
908 **PATIENT PROTECTION FROM ELECTRIC SHOCK**
909 **BREATHING APPARATUS WITH MEANS FOR PREVENTING PATIENT CROSS-CONTAMINATION**
910 **ANESTHESIA GAS SCAVENGING SYSTEM**
911 **UNILIMB INHALATION-EXHALATION BREATHING TUBES**
912 **CONNECTIONS AND CLOSURES FOR TUBES DELIVERING FLUIDS TO OR FROM THE BODY**
913 **BREATHABLE LIQUIDS**
914 **REBREATHING APPARATUS FOR INCREASING CARBON DIOXIDE CONTENT IN INHALED GAS**
915 **ULTRASOUND MAMMOGRAPHY**
916 **ULTRASOUND 3-D IMAGING**
917 **BODY FLUID, DEVICES FOR PROTECTION THEREFROM (E.G., AIDS, HEPATITUS, ETC.)**
918 .Condoms and shields
919 .Syringe, means to protect user
920 **COMPUTER ASSISTED MEDICAL DIAGNOSTICS**
921 .Diet management
922 .Including image analysis
923 .By comparison of patient data to other data
924 ..Using artificial intelligence
925 .Neural network

FOREIGN ART COLLECTIONS

FOR 000 **CLASS-RELATED FOREIGN DOCUMENTS**

DIGESTS

DIG 1 **MOTORIZED SYRINGE**
DIG 3 **HEART-LUNG**
DIG 6 **INTRAVENOUS INJECTION SUPPORT**
DIG 7 **SERVO-SYSTEMS**
DIG 8 **COLLAGEN**
DIG 10 **FLUID AMPLIFIERS**

DIG 12 PRESSURE INFUSION
DIG 13 INFUSION MONITORING
DIG 14 TEFLON
DIG 15 HOOK AND LOOP TYPE FASTENER
DIG 18 HEAT SHRINKABLE FILM
DIG 19 CLAVICLE SPLINT
DIG 20 INFLATABLE SPLINT
DIG 21 SILICONE
DIG 22 BLOOD COAGULATION
DIG 23 CERVICAL COLLARS
DIG 24 MEDICAL-SURGICAL BAGS
DIG 25 ARTIFICIAL SPHINETERS AND DEVICES FOR CONTROLLING URINARY INCONTINENCES
DIG 26 CANNULA SUPPORTERS
DIG 27 CRYOGENIC

Electronic Patent Application Fee Transmittal

Application Number:	11722018
Filing Date:	18-Jun-2007
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Filer:	Frank Christopher Eisenschenk/Jenny Bedner
Attorney Docket Number:	SER-125

Filed as Large Entity

U.S. National Stage under 35 USC 371 Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Claims in excess of 20	1615	28	52	1456
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 2 months with $0 paid	1252	1	490	490
Miscellaneous:				
			Total in USD ($)	**1946**

Electronic Acknowledgement Receipt

EFS ID:	6668020
Application Number:	11722018
International Application Number:	
Confirmation Number:	5532
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Customer Number:	23557
Filer:	Frank Christopher Eisenschenk/Jenny Bedner
Filer Authorized By:	Frank Christopher Eisenschenk
Attorney Docket Number:	SER-125
Receipt Date:	18-DEC-2009
Filing Date:	18-JUN-2007
Time Stamp:	13:50:53
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1946
RAM confirmation Number	10922
Deposit Account	190065
Authorized User	EISENSCHENK,FRANK C.

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Amd.pdf	2202869 205a2f67e512a338859e04a1b60d960cde106669	yes	26

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Claims	2	9
	Applicant Arguments/Remarks Made in an Amendment	10	26

Warnings:

Information:

2	Fee Worksheet (PTO-875)	fee-info.pdf	32469 10c9a9801379 71c78aef366f3331f72c686d7de5	no	2

Warnings:

Information:

Total Files Size (in bytes):	2235338

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
11/722,018	06/18/2007	

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT 12/18/2009	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 48	Minus	** 20	= 28	X $ =		OR	X $52=	1456
Independent (37 CFR 1.16(h))	* 3	Minus	***3	= 0	X $ =		OR	X $220=	0
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	**1456**

AMENDMENT	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/ANGELA D. JOHNSON/

I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on March 15, 2010.

Frank C. Eisenschenk, Ph.D., Patent Attorney

PETITION TO ADD INVENTORS
UNDER 37 C.F.R. §1.48(a)
Docket No. SER.125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Kimberly Ballard
Art Unit	:	1649
Applicant	:	Giampiero De Luca
Serial No.	:	11/722,018
Filed	:	June 18, 2007
Conf. No.	:	5532
For	:	Cladribine Regimen for Treating Multiple Sclerosis

Mail Stop PETITION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION UNDER 37 CFR §1.48(a)

Sir:

It is respectfully petitioned that the inventorship of the above-identified application be corrected to add Arnaud Ythier, Alain Munafo and Maria Lopez-Bresnahan as inventors. Authority for this petition and the correction of inventorship is found in 37 C.F.R. §1.48(a), reproduced below.

37 C.F.R. § 1.48 Correction of inventorship in a patent application, other than a reissue application

(a) If the inventive entity is set forth in error in an executed § 1.63 oath or declaration in a nonprovisional application, and such error arose without any deceptive intention on the part of the person named as an inventor in error or on the part of the person who through error was not named as an inventor, the inventorship of the nonprovisional application may be amended to name only the actual inventor or inventors. If the nonprovisional application is involved in an interference, the amendment must comply with the requirements of this section and must be accompanied by a motion under § 1.634. Amendment of the inventorship requires:

(1) A request to correct the inventorship that sets forth the desired inventorship change;

(2) A statement from each person being added as an inventor and from each person being deleted as an inventor that the error in inventorship occurred without deceptive intention on his or her part;

(3) An oath or declaration by the actual inventor or inventors as required by § 1.63 or as permitted by §§ 1.42, 1.43 or § 1.47;

(4) The processing fee set forth in § 1.17(i); and

(5) If an assignment has been executed by any of the original named inventors, the written consent of the assignee (see § 3.73(b) of this chapter).

Arnaud Ythier, Alain Munafo and Maria Lopez-Bresnahan were unintentionally, and without deceptive intent, not originally included on the application as co-inventors.

Accompanying this petition are:

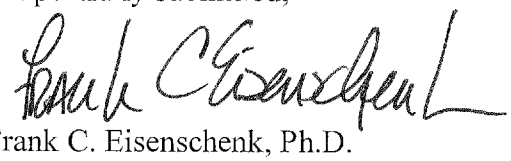
(1) A statement from the individuals being added as inventors that the error in inventorship occurred without deceptive intention on his/her part;

(2) A declaration under § 1.63 by the actual inventors; and

(3) The fee set forth in § 1.17(i).

The fee of $130.00 was paid at the time this Petition was filed. The Commissioner is also authorized to charge any additional fees that may be required by this paper to Deposit Account No. 19-0065.

Respectfully submitted,



Frank C. Eisenschenk, Ph.D.
Patent Attorney
Registration No. 45,332
Phone No.: (352) 375-8100
Fax No. : (352) 372-5800
Address : P.O. Box 142950
Gainesville, FL 32614-2950

FCE/sl
Attachments: as stated above

Docket No. SER.125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Giampiero De Luca
Serial No.	:	11/722,018
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P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT UNDER 37 C.F.R. §1.48(a)(2)

Sir:

As required by 37 C.F.R. §1.48(a)(2), the undersigned submits this paper to accompany the petition to correct inventorship filed on this same date and hereby states that the inventorship error excluding me, Arnaud Ythier, as a co-inventor on U.S. Serial No. 11/722,018 occurred without any deceptive intent on my part.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Name: Arnaud Ythier

July 15th 2009
Date

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Serial No.	:	11/722,018
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Alexandria, VA 22313-1450

STATEMENT UNDER 37 C.F.R. §1.48(a)(2)

Sir:

As required by 37 C.F.R. §1.48(a)(2), the undersigned submits this paper to accompany the petition to correct inventorship filed on this same date and hereby states that the inventorship error excluding me, Alain Munafo, as a co-inventor on U.S. Serial No. 11/722,018 occurred without any deceptive intent on my part.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Alain Munafo
Name: Alain Munafo

July 7, 2009
Date

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Docket No. SER.125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Serial No.	:	11/722,018
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Alexandria, VA 22313-1450

STATEMENT UNDER 37 C.F.R. §1.48(a)(2)

Sir:

As required by 37 C.F.R. §1.48(a)(2), the undersigned submits this paper to accompany the petition to correct inventorship filed on this same date and hereby states that the inventorship error excluding me, Maria Lopez-Bresnahan, as a co-inventor on U.S. Serial No. 11/722,018 occurred without any deceptive intent on my part.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Name: Maria Lopez-Bresnahan

Date 3/11/10

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Giampiero De Luca
Serial No.	:	11/722,018
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CONSENT UNDER 37 C.F.R. §1.48(a)(5)

Sir:

As required by 37 C.F.R. §1.48(a)(5), the undersigned, on behalf of Merck Serono SA, submits this paper and a certificate under 37 C.F.R. § 3.73(b) to accompany the petition to correct the inventorship for the above-referenced patent application. I hereby state that the assignee of record, Merck Serono SA, for Serial No. 11/722,018, agrees to the change of inventorship to add Arnaud Ythier, Alain Munafo and Maria Lopez-Bresnahan as co-inventors in the subject application as requested by the accompanying petition.

CERTIFICATE UNDER 37 CFR §3.73(b)

Merck Serono SA certifies that it is the assignee of the entire right, title, and interest in the patent application identified above by virtue of assignment from Giampiero De Luca to Laboratoires Serono, recorded in the United States Patent Office at REEL/FRAME 019685/0061 on August 13, 2007, and a Change of Name from Laboratoires Serono S.A. to Merck Serono, recorded in the United States Patent Office at REEL/FRAME 023000/0862 on July 24, 2009, and an assignment from Arnaud Ythier, Alain Munafo and Maria Lopez-Bresnahan to Merck Serono SA, a copy of which is attached.

As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owners to Merck Serono SA was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

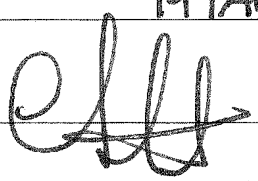
The undersigned has reviewed all of the documents in the chain of title of the patent application identified above and, to the best of undersigned's knowledge and belief, title is in the assignee named above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

MERCK SERONO SA

Date: 19 August 2009

Signature: 

Name: Giampiero De Luca

Title: Authorized Representative

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ASSIGNMENT

WHEREAS, we, the undersigned, residing at the indicated addresses given below, have invented certain new and useful improvements in **CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS**, for which an application for United States Letters Patent was filed June 18, 2007, as Serial No. 11/722,018.

WHEREAS, MERCK SERONO S.A., a corporation of the country of Switzerland, having a place of business at Centre Industriel, 1267 Coinsins, Vaud, Switzerland, is desirous of acquiring the entire right, title, and interest in and to said invention and in and to any Letters Patent which may be granted therefor in the United States and in any and all foreign countries;

NOW, THEREFORE, in view of MERCK SERONO S.A.'s review and evaluation of our patent disclosure and other valuable consideration, receipt of which is hereby acknowledged, I, the undersigned, have sold, assigned, and transferred, and by these presents do sell, assign, and transfer, unto said MERCK SERONO S.A., its successors and assigns, the full and exclusive right to the said invention in the United States and its territorial possessions and in all foreign countries and the entire right, title, and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations, and extensions thereof.

We hereby authorize and request the Patent Office Officials in the United States and in any and all foreign countries to issue any and all of said Letters Patent, when granted, to MERCK SERONO S.A., as the assignees of the entire right, title, and interest in and to the same, for the sole use and behoof of MERCK SERONO S.A., its successors and assigns.

FURTHER, we agree that we will communicate to MERCK SERONO S.A., or its representatives, any facts known to us respecting said invention; testify in any legal proceedings; sign all lawful papers; execute all divisional, continuation, substitution, renewal, and reissue applications; execute all necessary assignment papers to cause any and all of said Letters Patent to be issued to MERCK SERONO S.A.; make all rightful oaths; and generally do everything possible to aid MERCK SERONO S.A., its successors and assigns, to obtain and enforce proper protection for said invention in the United States and in any and all foreign countries.

IN TESTIMONY WHEREOF, I have hereunto set my hand this 15th day of July, 2009.

Signed _____

Arnaud Ythier
Route de Vireloup 88
1239 Collex-Bossy
Switzerland

WITNESS:

Signature: _____

Printed Name: CAROL JASCI

Date: 15.7.2009

IN TESTIMONY WHEREOF, I have hereunto set my hand this 07 day of July, 2009.

Signed Alain Munafo

Alain Munafo
Rue des Pressoirs 6
1180 Tartegnin
Switzerland

WITNESS:

Signature:

Printed Name: CECE FORSSMANN

Date: July 7th, 2009

IN TESTIMONY WHEREOF, I have hereunto set my hand this 11th day of March, 2010.

Signed _____

Maria Lopez-Bresnahan
145 South Great Road
Lincoln, MA 01773

WITNESS:

Signature: _____

Printed Name: Victoria Bresnahan

Date: 11- March - 2010

Application Information

Application Number::	11/722,018
Filing date::	06/18/2008
Application Type::	Regular (National Stage)
Subject Matter::	Utility
Suggested Classification::	None
Suggested Group Art Unit::	None
CD-ROM or CD-R?::	None
Number of CD disks::	None
Number of copies of CDs::	None
Sequence submission?::	No
Computer Readable Form?::	No
Number of Copies of CRF::	None
Title::	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
Attorney Docket Number::	SER.125
Request for Early Publication::	No
Request for Non-Publication::	No
Suggested Drawing Figure::	None
Total Drawing Sheets::	None
Small Entity?::	No
Petition included?::	No
Petition Type::	N/A
Secrecy Order in Parent Appl.?::	No

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	Italy
Status::	Unknown
Inventor One Given Name::	Giampiero
Family Name::	DE LUCA
City of Residence::	Conches/Geneva
Country of Residence::	Switzerland
Street of Mailing Address::	Chemin des Conches 15B
City of Mailing Address::	Conches/Geneva
Country of Mailing Address::	Switzerland
Postal or Zip Code of Mailing Address::	CH-1231

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	CH and FR
Status::	Unknown
Inventor Two Given Name::	Arnaud
Family Name::	YTHIER
City of Residence::	Collex-Bossy
Country of Residence::	Switzerland
Street of Mailing Address::	Route de Vireloup 88
City of Mailing Address::	Collex-Bossy
Country of Mailing Address::	Switzerland
Postal or Zip Code of Mailing Address::	1239

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	CH
Status::	Unknown
Inventor Three Given Name::	Alain
Family Name::	MUNAFO
City of Residence::	Tartegnin
Country of Residence::	Switzerland
Street of Mailing Address::	Rue des Pressoirs 6
City of Mailing Address::	Tartegnin
Country of Mailing Address::	Switzerland
Postal or Zip Code of Mailing Address::	1180

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	US
Status::	Unknown
Inventor Four Given Name::	Maria
Family Name::	LOPEZ-BRESNAHAN
City of Residence::	Lincoln
State or Province of Residence::	MA
Street of Mailing Address::	145 South Great Road
City of Mailing Address::	Lincoln
State or Province of mailing address::	MA
Postal or Zip Code of Mailing Address::	01773

Representative Information

Representative Customer Number::	000023557

Correspondence Information

Correspondence Customer Number::	000023557
Telephone Number One::	(352) 375-8100
Telephone Number Two::	
Fax Number::	(352) 372-5800
Electronic Mail Address::	fce@slspatents.com

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This application is a	National Stage of	PCT/EP2005/056954	December 20, 2005
PCT/EP2005/056954	An application claiming the benefit under 35 USC 119(e) of	60/638,669	December 22, 2004

Foreign Priority Information

Country::	Application Number::	Filing Date::	Priority Claimed::
EP	04106909.7	December 22, 2004	Yes

Assignee Information

Assignee Name::	~~Laboratoires Serono S.A.~~ Merck Serono S.A.
Street of Mailing Address::	~~Zone Industrielle de l'Ouriettaz~~ Centre Industriel
City of Mailing Address::	~~Aubonne~~ Coinsins, Vaude
Country of Mailing Address::	Switzerland
Postal or Zip Code of Mailing Address::	~~CH-1170~~ 1267

PTO/SB/01A (09-04)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	CLABRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☐ The attached application, or

☑ Application No. PCT/EP2005/056954, filed on DECEMBER 20, 2005,

☑ as amended on JUNE 18, 2007 (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

All statements made herein of my/own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: GIAMPIERO DE LUCA

Signature: _____ Citizen of: SWITZERLAND

Inventor two: ARNAUD YTHIER

Signature: _____ Citizen of: SWITZERLAND and FRANCE

Inventor three: ALAIN MUNAFO

Signature: _____ Citizen of: SWITZERLAND

Inventor four: MARIA LOPEZ-BRESNAHAN

Signature: _____ Citizen of: UNITED STATES

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

PTO/SB/01A (09-04)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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FULL NAME OF INVENTOR(S)

Inventor one: GIAMPIERO DE LUCA

Signature: _____ Citizen of: SWITZERLAND

Inventor two: ARNAUD YTHIER

Signature: _____ Citizen of: SWITZERLAND and FRANCE

Inventor three: ALAIN MUNAFO

Signature: _____ Citizen of: SWITZERLAND

Inventor four: MARIA LOPEZ-BRESNAHAN

Signature: _____ Citizen of: UNITED STATES

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

PTO/SB/01A (09-04)
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FULL NAME OF INVENTOR(S)

Inventor one: GIAMPIERO DE LUCA

Signature: _____ Citizen of: SWITZERLAND

Inventor two: ARNAUD YTHIER

Signature: _____ Citizen of: SWITZERLAND and FRANCE

Inventor three: ALAIN MUNAFO

Signature: _____ Citizen of: SWITZERLAND

Inventor four: MARIA LOPEZ-BRESNAHAN

Signature: _____ Citizen of: UNITED STATES

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/01A (09-04)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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FULL NAME OF INVENTOR(S)

Inventor one: GIAMPIERO DE LUCA

Signature: _____ Citizen of: SWITZERLAND

Inventor two: ARNAUD YTHIER

Signature: _____ Citizen of: SWITZERLAND and FRANCE

Inventor three: ALAIN MUNAFO

Signature: _____ Citizen of: SWITZERLAND

Inventor four: MARIA LOPEZ-BRESNAHAN

Signature: _____ Citizen of: UNITED STATES

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

PTO/SB/81 (01-09)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Application Number	11/722,018
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Title	Clabribind Regimen for ...
Art Unit	1614
Examiner Name	
Attorney Docket Number	SER.125

I hereby revoke all previous powers of attorney given in the above-identified application.

☒ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith: 23557

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified application to:

☒ The address associated with the above-mentioned Customer Number.

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☒ Applicant/Inventor.

OR

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____.

SIGNATURE of Applicant or Assignee of Record

Signature		Date	15th July 2009
Name	ARNAUD YTHIER	Telephone	
Title and Company			

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

PTO/SB/81 (01-09)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Application Number	11/722,018
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Title	Clabribind Regimen for ...
Art Unit	1614
Examiner Name	
Attorney Docket Number	SER.125

I hereby revoke all previous powers of attorney given in the above-identified application.

☒ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

23557

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified application to:

☒ The address associated with the above-mentioned Customer Number.

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☒ Applicant/Inventor.

OR

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____

SIGNATURE of Applicant or Assignee of Record

Signature	Alain Munafo	Date	July 7, 2009
Name	ALAIN MUNAFO	Telephone	+ 41 22 414 3833
Title and Company	Merck Serono S.A. Geneva		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/81 (01-09)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Application Number	11/722,018
Filing Date	June 18, 2007
First Named Inventor	Giampiero de Luca
Title	Clabribind Regimen for ...
Art Unit	1614
Examiner Name	
Attorney Docket Number	SER.125

I hereby revoke all previous powers of attorney given in the above-identified application.

☒ A Power of Attorney is submitted herewith.

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23557

OR

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Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified application to:

☒ The address associated with the above-mentioned Customer Number.

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☒ Applicant/Inventor.

OR

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____.

SIGNATURE of Applicant or Assignee of Record

Signature		Date	11-March-2010
Name	MARIA LOPEZ-BRESNAHAN	Telephone	
Title and Company			

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Patent Application Fee Transmittal

Application Number:	11722018
Filing Date:	18-Jun-2007
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Filer:	Frank Christopher Eisenschenk/Jenny Bedner
Attorney Docket Number:	SER-125

Filed as Large Entity

U.S. National Stage under 35 USC 371 Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Petition fee- 37 CFR 1.17(h) (Group III)	1464	1	130	130
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		**130**

Electronic Acknowledgement Receipt

EFS ID:	7206759
Application Number:	11722018
International Application Number:	
Confirmation Number:	5532
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Customer Number:	23557
Filer:	Frank Christopher Eisenschenk/Jenny Bedner
Filer Authorized By:	Frank Christopher Eisenschenk
Attorney Docket Number:	SER-125
Receipt Date:	15-MAR-2010
Filing Date:	18-JUN-2007
Time Stamp:	12:41:29
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$130
RAM confirmation Number	9250
Deposit Account	190065
Authorized User	EISENSCHENK,FRANK C.

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	Comm.pdf	93741 edda5c2a3413921285d2ca539ec7035c8600cdda	no	2
Warnings:					
Information:					
2	Petition for review by the Technology Center SPRE.	Petition.pdf	668293 13fcd3f5a28364e5b4183fca2998a7b9723cbb3d	no	11
Warnings:					
Information:					
3	Application Data Sheet	ADS-supp.pdf	242033 bfa9511b378c1352bf39bd1a30a15c09569e69b8	no	5
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
4	Oath or Declaration filed	executed-Dec-POA.pdf	1113099 9ae6df822a683499fc429ff6b7224cba986048ec	no	7
Warnings:					
Information:					
5	Fee Worksheet (PTO-875)	fee-info.pdf	30394 3fe8ba2da71ba6a58daf218b535364e557e3cd6d	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	2147560		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on March 15, 2010.

Frank C. Eisenschenk, Ph.D., Patent Attorney

COMMUNICATION
Docket No. SER.125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Kimberly Ballard
Art Unit	:	1649
Applicant	:	Giampiero De Luca
Serial No.	:	11/722,018
Filed	:	June 18, 2007
Conf. No.	:	5532
For	:	Cladribine Regimen for Treating Multiple Sclerosis

Mail Stop PETITION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

COMMUNICATION UNDER 37 CFR §1.48(a)

Sir:

Attached herewith are the following documents:

1) Petition Under 37 CFR §1.48(a);
2) Statement under 37 CFR §1.48(a)(2) submitted by added inventor Arnaud Ythier;
3) Statement under 37 CFR §1.48(a)(2) submitted by added inventor Alain Munafo;
4) Statement under 37 CFR §1.48(a)(2) submitted by added inventor Maria Lopez-Bresnahan;
5) Executed Declaration (37 CFR §1.63) form signed by all inventors;
6) Power of Attorney signed by Arnaud Ythier;
7) Power of Attorney signed by Alain Munafo;
8) Power of Attorney signed by Maria Lopez-Bresnahan;
9) Consent and Certificate Under 37 CFR §3.73(b); and
10) Supplemental Application Data Sheet.

J:\SER\125\Add Inventor\Comm.doc/DNB/sl

The Commissioner is authorized to charge any fees that may be required by this paper to Deposit Account No. 19-0065.

Respectfully submitted,

Frank C. Eisenschenk, Ph.D.
Patent Attorney
Registration No. 45,332
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

FCE/sl
Attachments: as stated above

J:\SER\125\Add Inventor\Comm.doc/DNB/sl

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

23557 7590 03/22/2010

SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO Box 142950
GAINESVILLE, FL 32614

EXAMINER	
BALLARD, KIMBERLY	
ART UNIT	PAPER NUMBER
1649	

DATE MAILED: 03/22/2010

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/722,018	06/18/2007	Giampiero De Luca	SER-125	5532

TITLE OF INVENTION: CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1510	$300	$0	$1810	06/22/2010

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

23557 7590 03/22/2010

SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO Box 142950
GAINESVILLE, FL 32614

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/722,018	06/18/2007	Giampiero De Luca	SER-125	5532

TITLE OF INVENTION: CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1510	$300	$0	$1810	06/22/2010

EXAMINER	ART UNIT	CLASS-SUBCLASS
BALLARD, KIMBERLY	1649	514-046000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/722,018	06/18/2007	Giampiero De Luca	SER-125	5532

23557 7590 03/22/2010

SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO Box 142950
GAINESVILLE, FL 32614

EXAMINER	
BALLARD, KIMBERLY	
ART UNIT	PAPER NUMBER
1649	

DATE MAILED: 03/22/2010

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 301 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 301 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Notice of Allowability	Application No.	Applicant(s)	
	11/722,018	DE LUCA, GIAMPIERO	
	Examiner	Art Unit	
	Kimberly Ballard	1649	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the response filed 18 December 2009*.

2. ☒ The allowed claim(s) is/are *18-25 and 27-66*.

3. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☒ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☒ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☒ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☒ Interview Summary (PTO-413), Paper No./Mail Date *20100311* .
7. ☒ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	11/722,018	DE LUCA, GIAMPIERO	
	Examiner	Art Unit	
	Kimberly Ballard	1649	

All Participants:

(1) *Kimberly Ballard*.

(2) *Chris Eisenschenk*.

Status of Application: *pending*

(3) _____.

(4) _____.

Date of Interview: *10 March 2010*

Time: *2 PM*

Type of Interview:
☒ Telephonic
☐ Video Conference
☐ Personal (Copy given to: ☐ Applicant ☐ Applicant's representative)

Exhibit Shown or Demonstrated: ☐ Yes ☒ No
If Yes, provide a brief description: .

Part I.

Rejection(s) discussed:

Claims discussed:
46

Prior art documents discussed:

Part II.

SUBSTANCE OF INTERVIEW DESCRIBING THE GENERAL NATURE OF WHAT WAS DISCUSSED:
Discussed minor examiner's amendments to claims. Discussed co-pending junior application with similar subject matter.

Part III.

☒ It is not necessary for applicant to provide a separate record of the substance of the interview, since the interview directly resulted in the allowance of the application. The examiner will provide a written summary of the substance of the interview in the Notice of Allowability.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview, since the interview did not result in resolution of all issues. A brief summary by the examiner appears in Part II above.

/Kimberly Ballard/
Examiner, Art Unit 1649

(Applicant/Applicant's Representative Signature – if appropriate)

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Chris Eisenschenk on March 10, 2010.

The application has been amended as follows:

IN THE CLAIMS

In claim 46, line 10 of the claim (step iv), replace the period "." at the end of the line with a semicolon -- ; -- .

Examiner's Notes

The art made of record and not relied upon is considered pertinent to applicant's disclosure: US 2010/0021429 by Brentzel et al. (published 01/28/2010; filed 05/23/2007). This patent application currently has the same assignee (Merck Serono SA) and has similar claimed subject matter, but is considered junior to the instant application.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably

accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Advisory Information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kimberly Ballard whose telephone number is 571-272-2150. The examiner can normally be reached on Monday-Friday 8:30 AM - 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Stucker can be reached on 571-272-0911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Kimberly Ballard
Art Unit 1649

/Elizabeth C. Kemmerer/
Elizabeth C. Kemmerer, Ph.D.
Primary Examiner, Art Unit 1646

Notice of References Cited	Application/Control No. 11/722,018	Applicant(s)/Patent Under Reexamination DE LUCA, GIAMPIERO	
	Examiner Kimberly Ballard	Art Unit 1649	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-2010/0021429	01-2010	Brentzel et al.	424/85.6
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L7	453	(cladribine leustatin (2-chlorodeoxyadenosine) (2-chloro-2'deoxyadenosine)).clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2010/03/08 15:16
L8	67201	multiple adj sclerosis	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2010/03/08 15:16
L9	101	l7 and L8	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2010/03/08 15:16

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L5	0	De-luca-gia$.in.	USPAT; UPAD	OR	ON	2010/03/08 15:05
L6	69	(cladribine leustatin (2-chlorodeoxyadenosine) (2-chloro-2'deoxyadenosine)).clm.	USPAT; UPAD	OR	ON	2010/03/08 15:06

3/8/2010 3:27:55 PM
C:\Documents and Settings\kballard\My Documents\EAST\Workspaces\11.722018.wsp

```
     (FILE 'HOME' ENTERED AT 15:32:38 ON 08 MAR 2010)

     FILE 'CAPLUS' ENTERED AT 15:32:55 ON 08 MAR 2010
                E US2007-722018/APPS
L1              1 S E3

     FILE 'STNGUIDE' ENTERED AT 15:33:26 ON 08 MAR 2010

     FILE 'REGISTRY' ENTERED AT 15:34:07 ON 08 MAR 2010
L2              1 S 4291-63-8/RN
                SET NOTICE 1 DISPLAY
                SET NOTICE LOGIN DISPLAY

     FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 15:34:51 ON 08 MAR 2010
L3         145708 S MULTIPLE(A)SCLEROSIS
L4            638 S L2 AND L3
L5              0 S L4 AND ((INDUCTION OR MAINTENANCE)(S)(PHASE OR PERIOD))
L6         369890 S (INDUCTION OR MAINTENANCE OR TREATMENT)(S)(PHASE OR PERIOD)
L7             41 S L4 AND L6
L8             25 DUP REM L7 (16 DUPLICATES REMOVED)
L9            906 S DE LUCA G/AU
L10            19 S L9 AND L3
L11            10 DUP REM L10 (9 DUPLICATES REMOVED)
```

Issue Classification

Application/Control No.	Applicant(s)/Patent Under Reexamination
11722018	DE LUCA, GIAMPIERO
Examiner	**Art Unit**
Kimberly A. Ballard	1649

ORIGINAL		INTERNATIONAL CLASSIFICATION									
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED				
514	46	A	6	1	K	31 / 52 (2006.01.01)	A	6	1	K	9 / 00 (2006.01.01)
CROSS REFERENCE(S)		A	6	1	K	31 / 7076 (2006.01.01)					
		A	6	1	K	38 / 21 (2006.01.01)					

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				
424	85.6				

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

/Kimberly A. Ballard/ Examiner.Art Unit 1649	03/11/2010	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	48	
/Elizabeth C. Kemmerer/ Primary Examiner.Art Unit 1646	03/12/2010	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	11722018	DE LUCA, GIAMPIERO
	Examiner	Art Unit
	Kimberly Ballard	1649

SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor search (PALM, EAST, NPL) - updated search	03/08/2010	KAB
EAST (USPAT, USOCR, PGPUB, DERWENT, FPRS, EPO, JPO) - updated search	03/08/2010	KAB
STN (MEDLINE, BIOSIS, CAPLUS, EMBASE) - updated search	03/08/2010	KAB
Patentability conference with Elizabeth Kemmerer (primary examiner) and Jeffrey Stucker (SPE)	03/10/2010	KAB

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner
	See EAST search results	03/08/2010	KAB

To: euspto@slspatents.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 23557

Mar 22, 2010 05:53:14 AM

Dear PAIR Customer:

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Application	Document	Mailroom Date	Attorney Docket No.
11722018	NOA	03/22/2010	SER-125
	EXIN	03/22/2010	SER-125
	NOA	03/22/2010	SER-125
	892	03/22/2010	SER-125

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Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/722,018	06/18/2007	Giampiero De Luca	SER.125	5532

23557 7590 03/25/2010

SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO Box 142950
GAINESVILLE, FL 32614

EXAMINER	
BALLARD, KIMBERLY	

ART UNIT	PAPER NUMBER
1649	

NOTIFICATION DATE	DELIVERY MODE
03/25/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
11722018	6/18/2007	DE LUCA, GIAMPIERO	SER-125

SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
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GAINESVILLE, FL 32614

EXAMINER
Kimberly Ballard

ART UNIT	PAPER
1649	20100316

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

In view of the papers filed March 15, 2010, it has been found that this nonprovisional application, as filed, through error and without deceptive intent, improperly set forth the inventorship, and accordingly, this application has been corrected in compliance with 37 CFR 1.48(a). The inventorship of this application has been changed by addition of Arnaud Ythier, Alain Munafo, and Maria Lopez-Bresnahan.

The application will be forwarded to the Office of Initial Patent Examination (OIPE) for issuance of a corrected filing receipt, and correction of Office records to reflect the inventorship as corrected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kimberly Ballard whose telephone number is 571-272-2150. The examiner can normally be reached on Monday-Friday 8:30 AM - 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Stucker can be reached on 571-272-0911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Elizabeth C. Kemmerer/
Primary Examiner, Art Unit 1646

I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on March 15, 2010.

Frank C. Eisenschenk, Ph.D., Patent Attorney

PETITION TO ADD INVENTORS
UNDER 37 C.F.R. §1.48(a)
Docket No. SER.125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Kimberly Ballard
Art Unit	:	1649
Applicant	:	Giampiero De Luca
Serial No.	:	11/722,018
Filed	:	June 18, 2007
Conf. No.	:	5532
For	:	Cladribine Regimen for Treating Multiple Sclerosis

Mail Stop PETITION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION UNDER 37 CFR §1.48(a)

Sir:

It is respectfully petitioned that the inventorship of the above-identified application be corrected to add Arnaud Ythier, Alain Munafo and Maria Lopez-Bresnahan as inventors. Authority for this petition and the correction of inventorship is found in 37 C.F.R. §1.48(a), reproduced below.

37 C.F.R. § 1.48 Correction of inventorship in a patent application, other than a reissue application

(a) If the inventive entity is set forth in error in an executed § 1.63 oath or declaration in a nonprovisional application, and such error arose without any deceptive intention on the part of the person named as an inventor in error or on the part of the person who through error was not named as an inventor, the inventorship of the nonprovisional application may be amended to name only the actual inventor or inventors. If the nonprovisional application is involved in an interference, the amendment must comply with the requirements of this section and must be accompanied by a motion under § 1.634. Amendment of the inventorship requires:

(1) A request to correct the inventorship that sets forth the desired inventorship change;

Please see petition under 37 CFR 1.48a received 15 March 2010 for addition of new inventors. /KAB/ 03/16/2010

PTO/SB/01A (09-04)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	CLABRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☐ The attached application, or

☑ Application No. PCT/EP2005/056954, filed on DECEMBER 20, 2005,

☑ as amended on JUNE 18, 2007 (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

All statements made herein of my/own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: GIAMPIERO DE LUCA

Signature: _____ Citizen of: SWITZERLAND

Inventor two: ARNAUD YTHIER

Signature: _____ Citizen of: SWITZERLAND and FRANCE

Inventor three: ALAIN MUNAFO

Signature: _____ Citizen of: SWITZERLAND

Inventor four: MARIA LOPEZ-BRESNAHAN

Signature: _____ Citizen of: UNITED STATES

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

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BIB DATA SHEET

CONFIRMATION NO. 5532

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/722,018	06/18/2007	514	1649	SER-125

APPLICANTS

Giampiero De Luca, Conches, SWITZERLAND; Arnaud Ythier, Collex-Bossy, SWITZERLAND; Alain Munafo, Tartegnin, SWITZERLAND; Maria Lopez-Bresnahan, Lincoln, MA.

**** CONTINUING DATA ***************************

This application is a 371 of PCT/EP2005/056954 12/20/2005
which claims benefit of 60/638,669 12/22/2004

**** FOREIGN APPLICATIONS ***************************

/KAB/ 03/16/2010

EUROPEAN PATENT OFFICE (EPO) 04106909.7 12/22/2004

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

12/10/2008

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☑ Yes ☐ No	☑ Yes ☐ No	/KIMBERLY BALLARD/ Examiner's Signature	☐ Initials	SWITZERLAND	0	20	1

ADDRESS

SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
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GAINESVILLE, FL 32614
UNITED STATES

TITLE

CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

FILING FEE RECEIVED		
2356	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

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Application	Document	Mailroom Date	Attorney Docket No.
11722018	NRES	03/25/2010	SER.125

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23557 7590 03/22/2010

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A PROFESSIONAL ASSOCIATION
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GAINESVILLE, FL 32614
Ph: (352)375-8100 Fax: (352)372-5800

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

N/A - Filed EFS	(Depositor's name)
	(Signature)
	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/722,018	06/18/2007	Giampiero De Luca	SER-125	5532

TITLE OF INVENTION: CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1510	$300	$0	$1810	06/22/2010

EXAMINER	ART UNIT	CLASS-SUBCLASS
BALLARD, KIMBERLY	1649	514-046000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 SALIWANCHIK, LLOYD & SALIWANCHIK
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

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(A) NAME OF ASSIGNEE: MERCK SERONO S.A.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): COINSINS, VAUD, SWITZERLAND

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

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☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

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Authorized Signature Frank C Eisenschenk Date March 25, 2010

Typed or printed name FRANK C. EISENSCHENK, PH.D. Registration No. 45,332

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Electronic Patent Application Fee Transmittal

Application Number:	11722018
Filing Date:	18-Jun-2007
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Filer:	Frank Christopher Eisenschenk/Jenny Bedner
Attorney Docket Number:	SER.125

Filed as Large Entity

U.S. National Stage under 35 USC 371 Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl issue fee	1501	1	1510	1510
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Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1810**

Electronic Acknowledgement Receipt

EFS ID:	7283753
Application Number:	11722018
International Application Number:	
Confirmation Number:	5532
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Customer Number:	23557
Filer:	Frank Christopher Eisenschenk/Jenny Bedner
Filer Authorized By:	Frank Christopher Eisenschenk
Attorney Docket Number:	SER.125
Receipt Date:	25-MAR-2010
Filing Date:	18-JUN-2007
Time Stamp:	13:20:02
Application Type:	U.S. National Stage under 35 USC 371

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	IssueFee.pdf	209402 c75551bf9ca7598464e3cb696dd4da3af272af93	no	1
Warnings:					
Information:					
2	Fee Worksheet (PTO-875)	fee-info.pdf	31967 2428e741ea520d0e40306892233567273b47befd	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	241369		

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National Stage of an International Application under 35 U.S.C. 371

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
11/722,018	06/18/2007	1649	2656	SER.125	20	1

CONFIRMATION NO. 5532

CORRECTED FILING RECEIPT

23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO Box 142950
GAINESVILLE, FL 32614

OC000000040889061

Date Mailed: 03/31/2010

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Giampiero De Luca, Conches, SWITZERLAND;
Arnaud Ythier, Collex-Bossy, SWITZERLAND;
Alain Munafo, Tartegnin, SWITZERLAND;
Maria Lopez-Bresnahan, Lincoln, MA;

Assignment For Published Patent Application

Laboratoires Serono S.A., Aubonne, SWITZERLAND

Power of Attorney: The patent practitioners associated with Customer Number 23557

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/EP2005/056954 12/20/2005
which claims benefit of 60/638,669 12/22/2004

Foreign Applications

EUROPEAN PATENT OFFICE (EPO) 04106909.7 12/22/2004

If Required, Foreign Filing License Granted: 12/10/2008

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 11/722,018**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title

CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

Preliminary Class

514

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

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Application	Document	Mailroom Date	Attorney Docket No.
11722018	APP.FILE.REC	03/31/2010	SER.125

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Bib Data Sheet

CONFIRMATION NO. 5532

SERIAL NUMBER	FILING OR 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/722,018	06/18/2007 RULE	514	1649	SER.125

APPLICANTS

Giampiero De Luca, Conches, SWITZERLAND;
Arnaud Ythier, Collex-Bossy, SWITZERLAND;
Alain Munafo, Tartegnin, SWITZERLAND;
Maria Lopez-Bresnahan, Lincoln, MA;

**** CONTINUING DATA ***************************

This application is a 371 of PCT/EP2005/056954 12/20/2005
which claims benefit of 60/638,669 12/22/2004

**** FOREIGN APPLICATIONS *********************

EUROPEAN PATENT OFFICE (EPO) 04106909.7 12/22/2004

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** 12/10/2008

Foreign Priority claimed ☐ yes ☐ no 35 USC 119 (a-d) conditions met ☐ yes ☐ no ☐ Met after Allowance Verified and Acknowledged _____ Examiner's Signature Initials	STATE OR COUNTRY SWITZERLAND	SHEETS DRAWING 0	TOTAL CLAIMS 20	INDEPENDENT CLAIMS 1

ADDRESS
23557

TITLE

CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

FILING FEE RECEIVED	
2656	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:

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11/722,018	06/18/2007 RULE	514	1649	SER.125

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Maria Lopez-Bresnahan, Lincoln, MA;

3-30-10

**** CONTINUING DATA ***************************

This application is a 371 of PCT/EP2005/056954 12/20/2005 which claims benefit of 60/638,669 12/22/2004

**** FOREIGN APPLICATIONS *********************

EUROPEAN PATENT OFFICE (EPO) 04106909.7 12/22/2004

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** 12/10/2008

Foreign Priority claimed	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☒ yes ☐ no	SWITZERLAND	0	20	1

35 USC 119 (a-d) conditions met: ☒ yes ☐ no ☐ Met after Allowance

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TITLE

CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

FILING FEE RECEIVED	FEES: Authority has been given in Paper
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/722,018	05/11/2010	7713947	SER.125	5532

23557 7590 04/21/2010

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ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 300 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Giampiero De Luca, Conches, SWITZERLAND;
Arnaud Ythier, Collex-Bossy, SWITZERLAND;
Alain Munafo, Tartegnin, SWITZERLAND;
Maria Lopez-Bresnahan, Lincoln, MA;

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Application	Document	Mailroom Date	Attorney Docket No.
11722018	ISSUE.NTF	04/21/2010	SER.125

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I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on May 20, 2010.

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. SER.125

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Giampiero De Luca, Arnaud Ythier, Alain Munafo, Maria Lopez-Bresnahan
Issued	:	May 11, 2010
Patent No.	:	7,713,947
Conf. No.	:	5532
For	:	Cladribine Regimen for Treating Multiple Sclerosis

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 1, line 31:	Page 1, lines 14-15:
"Clinical is disability"	--Clinical disability--

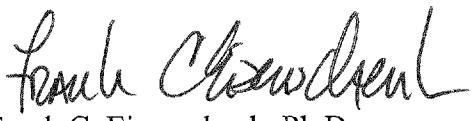
J:\SER\125\PTO-Misc\COC.Req.doc/DNB/jb

Column 7, line 32:	Page 12, lines 1-2:
"WFN-beta, WFN-beta"	--IFN-beta, IFN-beta--
Column 14, line 12:	Page 24, line 17:
"UI; (International unit)"	--UI (International unit)--.

A true and correct copy of pages 1, 12, and 24 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



Frank C. Eisenschenk, Ph.D.
Patent Attorney
Registration No. 45,332
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

FCE/jb
Attachments: Copy of pages 1, 12, and 24 of the specification
Certificate of Correction

Cladribine regimen for treating Multiple Sclerosis

Field of the Invention

The present invention relates to the use of multiple doses of Cladribine for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis.

Background of the Invention

Multiple sclerosis (MS) is the most known chronic inflammatory demyelinating disease of the central nervous system in humans. The onset of the disease typically occurs during ages 20 to 40. Women are affected approximately twice as often as men.

Over time, MS may result in the accumulation of various neurological disabilities. Clinical disability in MS is presumed to be a result of repeated inflammatory injury with subsequent loss of myelin and axons, leading to tissue atrophy.

MS is manifested in physical symptoms (relapses and disability progression), Central Nervous System (CNS) inflammation, brain atrophy and cognitive impairment. Presenting symptoms include focal sensory deficits, focal weakness, visual problems, imbalance and fatigue. Sexual impairment and sphincter dysfunction may occur. Approximately half of the patients with MS may experience cognitive impairment or depression.

MS is now considered to be a multi-phasic disease and periods of clinical quiescence (remissions) occur between exacerbations. Remissions vary in length and may last several years but are infrequently permanent.

Four courses of the disease are individualized: relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR) multiple sclerosis.

In one embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered during the Cladribine-free period.

In another embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered after the "treatment" according to the invention.

The term "interferon-beta (IFN-β)", as used herein, is intended to include fibroblast interferon in particular of human origin, as obtained by isolation from biological fluids or as obtained by DNA recombinant techniques from prokaryotic or eukaryotic host cells, as well as its salts, functional derivatives, variants, analogs and active fragments.

IFN-β suitable in accordance with the present invention is commercially available e.g. as Rebif® (Serono), Avonex® (Biogen) or Betaferon® (Schering). The use of interferons of human origin is also preferred in accordance with the present invention. The term interferon, as used herein, is intended to encompass salts, functional derivatives, variants, analogs and active fragments thereof.

Rebif® (recombinant human interferon-β) is the latest development in interferon therapy for multiple sclerosis (MS) and represents a significant advance in treatment. Rebif® is interferon (IFN)-beta 1a, produced from mammalian cell lines. It was established that interferon beta-1a given subcutaneously three times per week is efficacious in the treatment of Relapsing-Remitting Multiple Sclerosis (RRMS). Interferon beta-1a can have a positive effect on the long-term course of MS by reducing number and severity of relapses and reducing the burden of the disease and disease activity as measured by MRI.

The dosing of IFN-β in the treatment of relapsing-remitting MS according to the invention depends on the type of IFN-β used.

In accordance with the present invention, where IFN is recombinant IFN-β1b produced in E. Coli, commercially available under the trademark Betaseron®, it may preferably be

In another further embodiment, the invention provides a method according to the invention wherein the steps (iii) are repeated at least one or two times.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be administered in combination with interferon-beta.

Examples

The following abbreviations refer respectively to the definitions below:

kg (kilogram), **µg** (microgram), **mg** (milligram), **AEs** (Adverse effects), CNS (Cnetral nervous system), **CSF** (Cerebrospinal fluid), **EDSS** (Expanded Disability Status Scale, **SNRS** (Scripps Neurologic Rating Scale), **IFN** (interferon), **i.v.** (intra-veinous), **MIU** (Million International units), **MS** (multiple sclerosis), **MRI** (Magnetic resonance imaging), **p.o.** (per os), **PPMS** (Primary progressive multiple sclerosis), **PRMS** (Progressive relapsing multiple sclerosis), **RRMS** (Relapsing-remitting multiple sclerosis), **SPMS** (Secondary progressive multiple sclerosis), **s.c.** (subcutaneous), **TIW** (Three times a week), **2-CdA** (2-chloro-2'deoxyadenosine or Cladribine), **UI** (International unit).

The efficacy and safety of oral Cladribine administration, eventually multi-dose administration, according to the invention can be assessed for example following the protocol below:

Example 1: Oral cladribine in the treatment of relapsing forms of MS

A study of sixty patients with relapsing forms of clinically definite multiple sclerosis is undertaken. Each patient is first examined for normal hepatic, renal, and bone marrow functioning to establish baseline values.

Patients are selected from Male or Female, between 18 and 55 years of age who had one or more relapses within the prior 12 months. Female patients are non-pregnant female.

Patients are randomly assigned to one of the treatment groups listed in Table 1 below:

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,713,947

APPLICATION NO.: 11/722,018

DATED : May 11, 2010

INVENTORS : Giampiero De Luca, Arnaud Ythier, Alain Munafo, Maria Lopez-Bresnahan

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1,
Line 31, "Clinical is disability" should read --Clinical disability--.

Column 7,
Line 32, "WFN-beta, WFN-beta" should read --IFN-beta, IFN-beta--.

Column 14,
Line 12, "UI; (International unit)" should read --UI (International unit)--.

MAILING ADDRESS OF SENDER:
Saliwanchik, Lloyd & Saliwanchik
P.O. Box 142950
Gainesville, FL 32614-2950

Electronic Acknowledgement Receipt

EFS ID:	7651975
Application Number:	11722018
International Application Number:	
Confirmation Number:	5532
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Customer Number:	23557
Filer:	Frank Christopher Eisenschenk/Jenny Bedner
Filer Authorized By:	Frank Christopher Eisenschenk
Attorney Docket Number:	SER.125
Receipt Date:	20-MAY-2010
Filing Date:	18-JUN-2007
Time Stamp:	13:47:51
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Certificate of Correction	COC-Req.pdf	396206 9e7eef3d84f79fc56e6fac80984f434c5e166404	no	6

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,713,947 B2
APPLICATION NO. : 11/722018
DATED : May 11, 2010
INVENTOR(S) : Giampiero De Luca et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1,
Line 31, "Clinical is disability" should read --Clinical disability--.

Column 7,
Line 32, "WFN-beta, WFN-beta" should read --IFN-beta, IFN-beta--.

Column 14,
Line 12, "UI; (International unit)" should read --UI (International unit)--.

Signed and Sealed this

Fifteenth Day of June, 2010

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

This form is to be submitted with the Power of Attorney by Applicant Form to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form.

Application Number	11/722,018
Patent Number	7,713,947
Filing Date	June 18, 2007
Issue Date	May 11, 2010
First Named Inventor	Giampiero De Luca
Art Unit	1649
Examiner Name	BALLARD, KIMBERLY
Attorney Docket Number	000758US
Title	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

Signature of Applicant or Patent Practitioner

Signature	/Kirsten Grueneberg/
Name	Dr. Kirsten Grueneberg
Reg. No.	47,297
Customer No.	151167

Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

*Total of __1__ form(s) is/are submitted.

PTO/SB/81A (12-08)
Approved for use through 03/31/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT - POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Patent Number	7,713,947
Issue Date	May 11, 2010
First Named Inventor	Giampiero De Luca
Title	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
Attorney Docket No.	000758US

I hereby revoke all previous powers of attorney given in the above-identified patent.

☐ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the Customer Number identified in the box at right as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

151167

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified patent to:

☒ The address associated with the above-identified Customer Number.

OR

☐ The address associated with the Customer Number identified in the box at right:

OR

☐ Firm or Individual Name	
Address	
City	State / Zip
Country	
Telephone	Email

I am the:

☐ Inventor, having ownership of the patent.

OR

☒ Patent owner.

Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____

SIGNATURE of Inventor or Patent Owner

Signature		Date	May 31, 2011
Name	Pietro VON BALMOOS	Telephone	
Title and Company	Authorized Representative Merck Serono S.A.	Authorized Representative Merck Serono S.A.	

NOTE: Signatures of all the inventors or patent owners of the entire interest or their representative(s) are required. If more than one signature is required, submit multiple forms, check the box below, and identify the total number of forms submitted in the blank below.

☒ A total of 1 forms are submitted.

PTO/SB/96 (11-18)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Merck Serono S.A.

Application No./Patent No.: 7,713,947 Filed/Issue Date: May 11, 2010

Titled:
CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

Merck Serono S.A., a corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.

states that it is:

1. ☒ the assignee of the entire right, title, and interest in;

2. ☐ an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is _____ %); or

3. ☐ the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)

the patent application/patent identified above, by virtue of either:

A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel_____, Frame_____, or a copy* is attached.

OR

B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Giampiero DE LUCA To: LABORATOIRES SERONO S.A.
The document was recorded in the United States Patent and Trademark Office at
Reel 019685, Frame 0061, or a copy* is attached.

2. From: LABORATOIRES SERONO SA To: MERCK SERONO SA
The document was recorded in the United States Patent and Trademark Office at
Reel 023601, Frame 0156, or a copy* is attached.

3. From: Arnaud YTHIER; Alain MUNAFO; and Maria LOPEZ-BRESNAHAN To: MERCK SERONO S.A.
The document was recorded in the United States Patent and Trademark Office at
Reel 024080, Frame 0041, or a copy* is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ *As required by 37 CFR 3.73(b)(1)(i), if a copy/copies is/are attached, the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Kirsten Grueneberg/ May 21, 2019
Signature Date

Dr. Kirsten Grueneberg 47,297
Printed or Typed Name Title or Registration Number

Electronic Acknowledgement Receipt

EFS ID:	36070091
Application Number:	11722018
International Application Number:	
Confirmation Number:	5532
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Customer Number:	23557
Filer:	Eric J.I. Myers/Malika Ash Shakur
Filer Authorized By:	Eric J.I. Myers
Attorney Docket Number:	SER.125
Receipt Date:	21-MAY-2019
Filing Date:	18-JUN-2007
Time Stamp:	12:46:30
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		2019-05-21-POA-373-Stmt-as-filed.pdf	516944 87d826cb7901275f81b715972a46a76b6b985b1e	yes	3

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Power of Attorney	1	2
	Assignee showing of ownership per 37 CFR 3.73	3	3
Warnings:			
Information:			
	Total Files Size (in bytes):	516944	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
11/722,018	06/18/2007	Giampiero De Luca	SER.125

CONFIRMATION NO. 5532

POWER OF ATTORNEY NOTICE

23557
SALIWANCHIK, LLOYD & EISENSCHENK
A PROFESSIONAL ASSOCIATION
PO Box 142950
GAINESVILLE, FL 32614
UNITED STATES OF AMERICA

OC000000108485257

Date Mailed: 05/29/2019

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/21/2019.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/sibrahim/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
11/722,018	06/18/2007	Giampiero De Luca	000758US

CONFIRMATION NO. 5532

POA ACCEPTANCE LETTER

151167
Gruneberg and Myers PLLC
1775 Tysons Blvd
5th Floor
Tysons, VA 22102

OC000000108485613

Date Mailed: 05/29/2019

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/21/2019.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/sibrahim/

To: patent@gandmpatent.com,kg@gandmpatent.com,em@gandmpatent.com
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 151167

May 29, 2019 03:32:27 AM

Dear PAIR Customer:

Gruneberg and Myers PLLC
1775 Tysons Blvd
5th Floor
Tysons, VA 22102
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 151167 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
11722018	N570	05/29/2019	000758US
	N570	05/29/2019	000758US

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 06/07/2019

GARIAS SALE #00000001 Mailroom Dt: 05/24/2019 601920 11722018
01 FC : 1457 1,120.00 DA

Docket No. 000758US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT:	U.S. PAT. NO. 7,713,947
ISSUED:	MAY 11, 2010
APPLICATION:	11/722,018
FILED:	JUNE 18, 2007
INVENTORS:	DE LUCA ET AL.
EXPIRATION:	OCTOBER 16, 2026
TITLE:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

TRANSMITTAL LETTER

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Commissioner:

Enclosed are the following items for filing in connection with the above-referenced Patent and a Request for Extension of Patent Term under 35 U.S.C. §156:

1. Return Receipt Postcard, and
2. Three total copies (original plus two copies) of each of the following:

   This transmittal letter and certificate of mailing

   Fee Transmittal

   Application for Extension of Patent Term under 35 U.S.C. §156

   Exhibit A: U.S. Patent No. 7,713,947 ("the '947 patent")

   Exhibit B: Certificate of Correction of June 15, 2010 in the '947 patent

   Exhibit C: Assignment from inventor DE LUCA to LABORATORIES SERONO SA

Exhibit D: Assignment (Merger/Change of Name) from LABORATORIES SERONO SA to MERCK SERONO SA

Exhibit E: Assignment from inventors YTHIER, MUNAFO, and LOPEZ-BRESNAHAN to MERCK SERONO SA

Exhibit F: Letter from EMD Serono, Inc. of Billerica, MA, which is the Marketing Applicant for Mavenclad, authorizing Applicant Merck Serono SA to rely upon Marketing Applicant's activities

Exhibit G: Power of Attorney submitted May 21, 2019

Exhibit H: Mavenclad Label

Exhibit I: P.2.2 Drug Product information, submitted in NDA 22561 in May 2018, pages 20-32

Exhibit J: FDA Approval Letter for Mavenclad NDA 22561

Exhibit K: Email with Timestamp sent 4:42 pm on March 29, 2019 from Sandra Folkendt (FDA) to Tammy Sarnelli (Marketing Applicant)

Exhibit L: Leustatin (cladribine) NDA 020229: listing and label

Exhibit M: Maintenance Fee Statement (First Maintenance Fee Payment)

Exhibit N: Maintenance Fee Statement (Second Maintenance Fee Payment)

Exhibit O: Letter from FDA showing date of IND application 74634

Exhibit P: Letter signed October 13, 2009 from FDA to Marketing Applicant (receipt of NDA 22561)

Exhibit Q: Letter signed August 22, 2011 from FDA to Marketing Applicant (receipt of withdrawal of NDA 22561)

Exhibit R: Letter signed June 13, 2018 from FDA to Marketing Applicant (receipt of NDA 22561)

Exhibit S: Chronology of Major Communications between FDA and Marketing Applicant (for IND application 74634 and NDA 22561)

Please charge additional fee(s) or underpayment of fee(s) to Deposit Account No. 601920 under 37 CFR 1.16 and 1.17, and please credit any overpayment of fee(s) to Deposit Account No. 601920.

Respectfully Submitted,
GRÜNEBERG AND MYERS PLLC

Customer Number
151167
Phone: (571) 458-7790
Fax: (571) 458-7789

Dr. Kirsten Grueneberg
Attorney of Record
Registration No. 47,297

Eric Myers
Registration No. 68,546

CERTIFICATE OF MAILING

I hereby certify that this correspondence (along with any paper referred to as being attached or enclosed) and fee is being deposited with the United States Postal Service with sufficient postage as Priority Mail Express® with label number EJ 028 863 791 US in an envelope addressed to:

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

on May 24, 2019.

Signature /Kirsten Grueneberg/
Dr. Kirsten Grueneberg
Reg. No. 47,297

FEE TRANSMITTAL		
	Patent Number	7,713,947
	Issue Date	May 11, 2010
	First Named Inventor	Giampiero De Luca
☐No fee required	Application Number	11/722,018
	Filing Date	June 18, 2007
☒Total payment $ 1120.00	Attorney Docket No.	000758US
	Title	Cladribine regimen for treating multiple sclerosis

☐Applicant asserts small entity status, 37 CFR 1.27
☐Applicant asserts micro entity status, 37 CFR 1.29 *(Form PTO/SB/15 or equivalent enclosed or already submitted)*
☐Track 1 Prioritized Examination

Claims Fees:

Total:	(- 20) ×	$100	$
Independent:	(- 3) ×	$460	$
☐Multiple dependency		$820	$
☐Late filing declaration		$160	$
☐Non-electronic filing fee		$400	$
☐Non-English translation		$140	$

Extension Fees under 37 CFR 1.136(a) and 1.17(a), see petition filed herewith, if applicable:

☐Within first month	$ 200	$
☐Within second month	$ 600	$
☐Within third month	$1400	$
☐Within fourth month	$2200	$
☐Within fifth month	$3000	$

☐Terminal Disclaimer	$ 160	$
☐RCE – 1st Request	$1300	$
☐RCE – 2nd or Subseq.	$1900	$
☐Notice of Appeal	$ 800	$
☐Appl'n Size (pp.-100)/50 ×	$ 400	$

Other:
☒Extension of term of patent under §156 (fee under 1.20(j)(1)) $1120.00

Payment in the amount of $ 1120.00 paid by:
☐Credit Card (online if electronically filed, or attached if paper filed)
☒Deposit Account No. **601920**.

☒ Please charge additional fee(s) or underpayment of fee(s) to Deposit Account No. **601920** under 37 CFR 1.16 and 1.17, and please credit any overpayment of fee(s) to Deposit Account No. **601920**.

☒ If these papers are not considered timely, then Applicants hereby petition under 37 CFR 1.136 for any necessary extension of time, further authorizing any necessary extension of time fees to be charged to Deposit Account No. **601920**.

Respectfully Submitted,
GRÜNEBERG AND MYERS PLLC

Dr. Kirsten Gruneberg
Registration No. 47,297

Eric Myers
Registration No. 68,546

Customer Number
151167
Phone: (571) 458-7790
Fax: (571) 458-7789

Docket No. 000758US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT:	U.S. PAT. NO. 7,713,947
ISSUED:	MAY 11, 2010
APPLICATION:	11/722,018
FILED:	JUNE 18, 2007
INVENTORS:	DE LUCA ET AL.
EXPIRATION:	OCTOBER 16, 2026
TITLE:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

APPLICATION FOR EXTENSION OF TERM UNDER 35 USC §156
FOR U.S. PATENT NO. 7,713,947

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Commissioner:

Pursuant to 35 U.S.C. §156 and 37 C.F.R. §§1.710-1.791, Merck Serono SA of Coinsins, Switzerland ("Applicant") respectfully requests extension of the patent term of U.S. Patent No. 7,713,947 ("the '947 patent," Exhibit A, with Certificate of Correction, Exhibit B), issued on May 11, 2010, due to regulatory review.

Applicant is the owner and assignee of the entire right, title, and interest in the '947 patent. Inventor De Luca assigned his right, title, and interest to Laboratories Serono SA in an assignment executed on July 12, 2007 and recorded at reel/frame 019685/0061 (Exhibit C).[1] Laboratories Serono SA then changed to Merck Serono SA, assigning its interest to the same

[1] Other documents pertaining to corporate name changes were recorded, which had been executed prior to the assignment of Inventor De Luca to Laboratories Serono SA. Because they predate the assignment of any inventor, they are not relevant here. Additionally, further documents were recorded as set forth herein to document corporate name change, which further documents were executed after the assignment from Inventor De Luca to Laboratories Serono SA.

on December 12, 2008, as recorded at reel/frame 023601/0156 (Exhibit D). Remaining inventors Ythier, Munafo, and Lopez-Bresnahan then assigned their respective rights, titles, and interests to Merck Serono SA in assignments executed on July 15, 2009; July 7, 2009; and March 10, 2010, respectively, all of which are recorded at reel/frame 024080/0041 (Exhibit E).

The Approved Product relevant to this application is Mavenclad (cladribine) ("Mavenclad" or "Approved Product"). The Marketing Applicant for Mavenclad is EMD Serono, Inc. of Billerica, MA. A letter on behalf of the Marketing Applicant authorizing Applicant Merck Serono SA to rely upon the activities of the Marketing Applicant, its predecessors, and affiliates is attached hereto at Exhibit F.

Enclosed as Exhibit G is a copy of the Power of Attorney submitted in the '947 patent file on May 21, 2019, appointing the undersigned and other attorneys at the undersigned's law firm as agent to transact all business with the USPTO on behalf of Applicant in connection with the '947 patent.

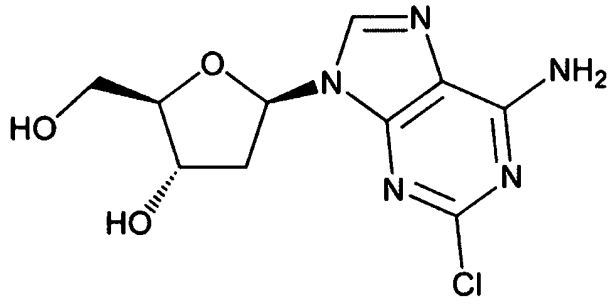
Applicant respectfully submits the following information in accordance with 35 U.S.C. §156 and 37 C.F.R. §§1.710-1.791. The following sections are numbered to correspond to the numbered subsections of 37 C.F.R. §1.740(a).

37 C.F.R. §1.740(a)(1): A complete identification of the approved product as by appropriate chemical and generic name, physical structure or characteristics

The Approved Product is Mavenclad. Mavenclad contains cladribine, hydroxypropyl betadex, magnesium stearate, and sorbitol. It is indicated for the treatment of relapsing forms of multiple sclerosis (MS), to include relapsing-remitting disease and active secondary progressive disease, in adults (Exhibit H, at pages 3 and 19-20).

Cladribine, a nucleoside metabolic inhibitor, is a white or almost white, non-hydroscopic, crystalline powder with the molecular formula $C_{10}H_{12}ClN_5O_3$ and molecular

weight 285.69. It differs in structure from the naturally occurring nucleoside, deoxyadenosine, by the substitution of chlorine for hydrogen in the 2-position of the purine ring. The chemical name of cladribine is 2-chloro-2′-deoxy-adenosine. The structural formula is shown below:



(Exhibit H, at pages 19-20).

Hydroxypropyl betadex (hydroxypropyl beta-cyclodextrin) forms complexes with various compounds. (Exhibit H at page 23). In several formulations in the Pharmacokinetic Studies and Clinical Trials leading to approval of Mavenclad, and in the commercial product of Mavenclad, the cladribine and cyclodextrin were and are present in the form of a complex. (Exhibit I, P.2.2 Drug Product information, submitted in NDA 22561 in May 2018, pages 20-32).

The Approved Product is available as 10 mg uncoated tablets. The tablets are white, round, biconvex, and engraved with a "C" on one side and "10" on the other side. The tablets are packaged in a blister. (Exhibit H, at pages 6-7).

37 C.F.R. §1.740(a)(2): A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred

Regulatory review of the Approved Product occurred under Section 505(b)(1) of the Federal Food, Drug, and Cosmetic Act (FDCA) (21 U.S.C. §355(b)).

37 C.F.R. §1.740(a)(3): An identification of the date on which the product received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred

The Approved Product received permission for commercial marketing or use under Section 505(b)(1) of the FDCA (21 U.S.C. §355(b)) on April 1, 2019.

35 U.S.C. §156(d)(1) states that "For purposes of determining the date on which a product receives permission under the second sentence of this paragraph, if such permission is transmitted after 4:30 P.M., Eastern Time, on a business day, or is transmitted on a day that is not a business day, the product shall be deemed to receive such permission on the next business day." (emphasis added). The Letter from the FDA to the Marketing Applicant granting permission for commercial marketing or use under Section 505(b)(1) of the FDCA (Exhibit J) was signed on Friday, March 29, 2019 at 4:27 pm. However, this letter was first transmitted to the Marketing Applicant on Friday, March 29, 2019 at 4:42 pm, as shown by the Timestamped Email from Sandra Folkendt (FDA) to Tammy Sarnello (Marketing Applicant). (Exhibit K). Accordingly, the Approved Product is deemed to have received permission on the next business day, which was Monday, April 1, 2019.

37 C.F.R. §1.740(a)(4): In the case of a drug product, an identification of each active ingredient in the product and as to each active ingredient, a statement that it has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved.

The active ingredient in the Approved Product is a cladribine-cyclodextrin complex. In several formulations in the Pharmacokinetic Studies and Clinical Trials leading to approval of Mavenclad, and in the commercial product of Mavenclad, the cladribine and cyclodextrin were and are present in the form of a complex. (Exhibit I, P.2.2 Drug Product information, submitted NDA 22561 in May 2018, pages 20-32).

A cladribine-cyclodextrin complex has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.

While cladribine-containing formulations have previously been approved under Section 505(b) of the FDCA, including an earliest approval in 1993 under the name Leustatin for treatment of hairy cell leukemia, Applicant notes that such approval was not for a product containing a cladribine-cyclodextrin complex as the active ingredient. (Exhibit L, listing and label for Leustatin (cladribine) NDA 020229 without inclusion of cyclodextrin).

37 C.F.R. §1.740(a)(5): A statement that the application is being submitted within the sixty day period permitted for submission pursuant to § 1.720(f) and an identification of the date of the last day on which the application could be submitted

This application is being submitted within the sixty-day period permitted for submission pursuant to § 1.720(f). Specifically, the sixty-day period beginning on April 1, 2019 ends on May 30, 2019, which is therefore the date of the last day on which the present application could be submitted.

37 C.F.R. §1.740(a)(6): A complete identification of the patent for which an extension is being sought by the name of the inventor, the patent number, the date of issue, and the date of expiration

Applicant seeks extension for the following (see Exhibit A):

U.S. Patent No.	7,713,947
Names of Inventors:	Giampiero De Luca, Arnaud Ythier, Alain Munafo, and Maria Lopez-Bresnahan
Date of Issue:	May 11, 2010
Date of Expiration:	October 16, 2026

As to the date of expiration, the '947 patent issued from U.S. Patent Application No. 11/722,018, filed on June 18, 2007, which was a national stage entry under 35 U.S.C. §371 of

PCT/EP2005/056954, filed on December 20, 2005.[2] The '947 patent is entitled to 300 days of Patent Term Adjustment, as indicated on the face of the patent itself. Thus, prior to the addition of the Patent Term Extension applied for herein, the term of the '947 patent extends twenty years plus 300 days from its international filing date. The '947 patent therefore expires on October 16, 2026.

37 C.F.R. §1.740(a)(7): A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings

A complete copy of the '947 patent is provided herewith as Exhibit A.

37 C.F.R. §1.740(a)(8): A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate issued in the patent

No terminal disclaimer or reexamination certificate has been issued. A certificate of correction, issued on June 15, 2010, is attached here as Exhibit B. Maintenance Fee Payment Statements are attached here: a statement for the payment of the first maintenance fee (Exhibit M) and a statement for the payment of the second maintenance fee (Exhibit N). The required maintenance fees which have been due, i.e. the first and second such fees, have been paid.

37 C.F.R. §1.740(a)(9): A statement that the patent claims the approved product, or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which at least one such patent claim reads on ... (ii) The method of using the approved product, if the listed claims include any claim to the method of using the approved product ...

The '947 patent claims a method of treatment of multiple sclerosis (MS) by oral administration of the Approved Product. At least claims 1-3, 6, 7, 9, 11, 16, 36, 38, 39, and 41-46 read on administration of the Approved Product for an approved use thereof, as shown below:

[2] The '947 patent also contains earlier claims to priority under 35 U.S.C. §119, but "Priority under section 119, 365(a), 365(b), 386(a), or 386(b) shall not be taken into account in determining the term of a patent." (35 U.S.C. §154(a)(3)).

Claim 1	Mavenclad Label (Exhibit H)
A method of treating multiple sclerosis	"MAVENCLAD is ... indicated for the treatment of relapsing forms of multiple sclerosis (MS)"
comprising the oral administration of a formulation comprising cladribine	"MAVENCLAD® (cladribine) tablets, for oral use"
wherein the formulation is to be orally administered following the sequential steps below: (i) an induction period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;	"The recommended cumulative dosage of MAVENCLAD is 3.5 mg per kg body weight administered orally and divided into 2 yearly treatment courses (1.75 mg per kg per treatment course)" "First Course/First Cycle: start any time." "First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"
(ii) a cladribine-free period of between about 8 and about 10 months wherein no cladribine formulation is administered; (iii) a maintenance period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the maintenance period is lower than the total dose of cladribine reached at the end of the induction period (i); and	"The recommended cumulative dosage of MAVENCLAD is 3.5 mg per kg body weight administered orally and divided into 2 yearly treatment courses (1.75 mg per kg per treatment course)" "Second Course/First Cycle: administer at least 43 weeks after the last dose of First Course/Second Cycle." "Second Course/Second Cycle: administer 23 to 27 days after the last dose of Second Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"
(iv) a cladribine-free period wherein no cladribine formulation is administered.	"Following the administration of 2 treatment courses, do not administer additional MAVENCLAD treatment during the next 2 years."

Claim 2	Mavenclad Label (Exhibit H)
The method according to claim 1,	See above
wherein the induction period lasts up to about 4 months.	"First Course/First Cycle: start any time." "First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"

Claim 3	Mavenclad Label (Exhibit H)
The method according to claim 2,	See above
wherein the induction period lasts about 2 months.	"First Course/First Cycle: start any time." "First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"

Claim 6	Mavenclad Label (Exhibit H)
The method according to claim 2,	See above
wherein the induction period lasts up to about 3 months.	"First Course/First Cycle: start any time." "First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"

Claim 7	Mavenclad Label (Exhibit H)
The method according to claim 1,	See above
wherein the total dose of cladribine reached at the end of the induction period is about 1.7 mg/kg.	"The recommended cumulative dosage of MAVENCLAD is 3.5 mg per kg body weight administered orally and divided into 2 yearly treatment courses (1.75 mg per kg per treatment course)"

Claim 9	Mavenclad Label (Exhibit H)
The method according to claim 1,	See above
wherein the cladribine-free (iv) period lasts about 10 months.	"Following the administration of 2 treatment courses, do not administer additional MAVENCLAD treatment during the next 2 years."

Claim 11	Mavenclad Label (Exhibit H)
The method according to claim 1,	See above
wherein the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg.	"The recommended cumulative dosage of MAVENCLAD is 3.5 mg per kg body weight administered orally and divided into 2 yearly treatment courses (1.75 mg per kg per treatment course)"

Claim 16	Mavenclad Label (Exhibit H)
The method according to claim 1,	See above
wherein the formulation is orally administered 1 to 7 days per month during the induction period.	"First Course/First Cycle: start any time." "First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"

(listing of claims continued on next page)

Claim 36	Mavenclad Label (Exhibit H)
A method of treating multiple sclerosis	"MAVENCLAD is ... indicated for the treatment of relapsing forms of multiple sclerosis (MS)"
comprising the oral administration of a formulation comprising cladribine	"MAVENCLAD® (cladribine) tablets, for oral use"
following the sequential steps below: (i) an induction period lasting from about 2 months to about 4 months wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;	"The recommended cumulative dosage of MAVENCLAD is 3.5 mg per kg body weight administered orally and divided into 2 yearly treatment courses (1.75 mg per kg per treatment course)" "First Course/First Cycle: start any time." "First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"
(ii) a cladribine-free period lasting from about 8 months to about 10 months, wherein no cladribine is administered; (iii) a maintenance period lasting from about 2 months to about 4 months, wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg;	"The recommended cumulative dosage of MAVENCLAD is 3.5 mg per kg body weight administered orally and divided into 2 yearly treatment courses (1.75 mg per kg per treatment course)" "Second Course/First Cycle: administer at least 43 weeks after the last dose of First Course/Second Cycle." "Second Course/Second Cycle: administer 23 to 27 days after the last dose of Second Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"
(iv) a cladribine-free period wherein no cladribine is administered.	"Following the administration of 2 treatment courses, do not administer additional MAVENCLAD treatment during the next 2 years."

Claim 38	Mavenclad Label (Exhibit H)
The method according to claim 36,	See above
wherein the induction period lasts about 2 months.	"First Course/First Cycle: start any time." "First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"

Claim 39	Mavenclad Label (Exhibit H)
The method according to claim 36,	See above
wherein the total dose of cladribine reached at the end of the induction period is about 1.7 mg/kg.	"The recommended cumulative dosage of MAVENCLAD is 3.5 mg per kg body weight administered orally and divided into 2 yearly treatment courses (1.75 mg per kg per treatment course)"

Claim 41	Mavenclad Label (Exhibit H)
The method according to claim 36,	See above
wherein the cladribine-free period (ii) lasts about 10 months.	"Second Course/First Cycle: administer at least 43 weeks after the last dose of First Course/Second Cycle."

Claim 42	Mavenclad Label (Exhibit H)
The method according to claim 36,	See above
wherein the cladribine-free (iv) period lasts 10 months.	"Following the administration of 2 treatment courses, do not administer additional MAVENCLAD treatment during the next 2 years."

Claim 43	Mavenclad Label (Exhibit H)
The method according to claim 36,	See above
wherein the maintenance period lasts about 2 months.	"Second Course/First Cycle: administer at least 43 weeks after the last dose of First Course/Second Cycle." "Second Course/Second Cycle: administer 23 to 27 days after the last dose of Second Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"

Claim 44	Mavenclad Label (Exhibit H)
The method according to claim 36,	See above
wherein the formulation is orally administered at a daily dose of 3 to 30 mg cladribine.	"10 mg Tablets" "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"

Claim 45	Mavenclad Label (Exhibit H)
The method according to claim 36,	See above
wherein the formulation is orally administered at a daily dose of 10 mg cladribine.	"10 mg Tablets" "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"

Claim 46	Mavenclad Label (Exhibit H)
The method according to claim 36,	See above
wherein the formulation is orally administered 1 to 7 days per month during the induction period.	"First Course/First Cycle: start any time." "First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle." "Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days"

* * *

37 C.F.R. §1.740(a)(10): A statement beginning on a new page of the relevant dates and information pursuant to 35 U.S.C.156(g) in order to enable the Secretary of Health and Human Services or the Secretary of Agriculture, as appropriate, to determine the applicable regulatory review period as follows:

(i) For a patent claiming a human drug, antibiotic, or human biological product:

The '947 patent, for which extension of term is sought, claims the use of a human drug.

(A) The effective date of the investigational new drug (IND) application and the IND number;

Approval of the Approved Product resulted from the filing of investigational new drug (IND) application 74634, which has an effective date of April 12, 2006, thirty days after the IND application receipt date of March 13, 2006. (Exhibit O).

(B) The date on which a new drug application (NDA) or a Product License Application (PLA) was initially submitted and the NDA or PLA number; and

Approval of the Approved Product resulted from the filing of New Drug Application (NDA) number 22561. As noted in the letter granting approval of the NDA, NDA 22561 has an original submission date of September 30, 2009. (Exhibit J at page 1). Applicants also submit herewith the following three letters from the Food and Drug Administration to the Marketing Applicant, evidencing the timeline of the NDA: Letter signed October 13, 2009 (receipt of NDA 22561, Exhibit P), Letter signed August 22, 2011 (receipt of withdrawal of NDA 22561, Exhibit Q), and Letter signed June 13, 2018 (receipt of NDA 22561, Exhibit R).

(C) The date on which the NDA was approved or the Product License issued

The NDA was approved on March 29, 2019. (Exhibit J). However, the Approved Product is deemed to have received permission on the next business day, which was Monday, April 1, 2019.

35 U.S.C. §156(d)(1) states that "For purposes of determining the date on which a product receives permission under the second sentence of this paragraph, if such permission

is transmitted after 4:30 P.M., Eastern Time, on a business day, or is transmitted on a day that is not a business day, the product shall be deemed to receive such permission on the next business day." (emphasis added). The Letter from the FDA to the Marketing Applicant granting permission for commercial marketing or use under Section 505(b)(1) of the FDCA (Exhibit J) was signed on Friday, March 29, 2019 at 4:27 pm. However, this letter was first transmitted to the Marketing Applicant on Friday, March 29, 2019 at 4:42 pm, as shown by the Timestamped Email from Sandra Folkendt (FDA) to Tammy Sarnello (Marketing Applicant). (Exhibit K). Accordingly, the Approved Product is deemed to have received permission on the next business day, which was Monday, April 1, 2019.

* * *

37 C.F.R. §1.740(a)(11): A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities

The brief description of the significant activities undertaken by the Marketing Applicant during the applicable regulatory review period with respect to the Approved Product and the significant dates applicable to such activities is provided herewith in Exhibit S: the Chronology of Major Communications between FDA and Marketing Applicant for IND application 74634 and NDA 22561. Applicants also submit herewith the following three letters from the Food and Drug Administration to the Marketing Applicant, evidencing the timeline of the NDA: Letter signed October 13, 2009 (receipt of NDA 22561, Exhibit P), Letter signed August 22, 2011 (receipt of withdrawal of NDA 22561, Exhibit Q), and Letter signed June 13, 2018 (receipt of NDA 22561, Exhibit R).

* * *

37 C.F.R. §1.740(a)(12): A statement beginning on a new page that in the opinion of the applicant the patent is eligible for the extension and a statement as to the length of extension claimed, including how the length of extension was determined

In the opinion of the Applicant, the '947 patent is eligible for the extension of 1826 days sought, having satisfied the requirements of 35 U.S.C. §156 and 37 C.F.R. §1.720 as follows:

35 U.S.C. §156(a), 37 C.F.R. §1.720(a): [the] patent ... claims a product, a method of using a product, or a method of manufacturing a product

The '947 patent claims a method of using a product.

35 U.S.C. §156(a)(1) , 37 C.F.R. §1.720(g): the term of the patent has not expired before an application is submitted under subsection (d)(1) for its extension

As noted above, the '947 patent (prior to patent term extension) expires on October 16, 2026, and thus has not yet expired before the submission of this application.

35 U.S.C. §156(a)(2), 37 C.F.R. §1.720(b): the term of the patent has never been extended under 35 U.S.C. §156(e)(1)

The term of the '947 patent has never been extended under 35 U.S.C. §156(e)(1).

35 U.S.C. §156(a)(3), 37 C.F.R. §1.720(c): an application for extension is submitted by the owner of record of the patent or its agent and in accordance with the requirements of 35 U.S.C. §156(d) and 37 C.F.R. §1.740

The present application for extension is submitted by the authorized attorney of the owner of record of the patent in accordance with the requirements of 35 U.S.C. §156(d) and 37 C.F.R. §1.740. (Exhibit G).

35 U.S.C. §156(a)(4), 37 C.F.R. §1.720(d): the product has been subject to a regulatory review period before its commercial marketing or use

The Approved Product, Mavenclad, was subject to a regulatory review period under section 505(b)(1) of the FDCA before its commercial marketing or use.

35 U.S.C. §156(a)(5)(A), 37 C.F.R. §1.720(e)(1): the permission for the commercial marketing or use of the product after such regulatory review period is the first permitted commercial marketing or use of the product under the provision of law under which such regulatory review period occurred

The active ingredient in the Approved Product is a cladribine-cyclodextrin complex. In several formulations in the Pharmacokinetic Studies and Clinical Trials leading to approval of Mavenclad, and in the commercial product of Mavenclad, the cladribine and cyclodextrin were and are present in the form of a complex. (Exhibit I, P.2.2 Drug Product information, submitted in NDA 22561 in May 2018, pages 20-32).

NDA 22561 is the first permitted commercial marketing or use of a cladribine-cyclodextrin complex under the Federal Food, Drug, and Cosmetic Act.

While cladribine-containing formulations have previously been approved under Section 505(b) of the FDCA, including an earliest approval in 1993 under the name Leustatin for treatment of hairy cell leukemia, Applicant notes that such approval was not for a product containing a cladribine-cyclodextrin complex as the active ingredient. (Exhibit L, listing and label for Leustatin (cladribine) NDA 020229 without inclusion of cyclodextrin).

37 C.F.R. §1.720(f): the application is submitted within the sixty-day period beginning on the date the product first received permission for commercial marketing or use under the provisions of law under which the applicable regulatory review period occurred

This application is being submitted within the sixty-day period permitted for submission pursuant to § 1.720(f). Specifically, the sixty-day period beginning on April 1, 2019 ends on May 30, 2019, which is therefore the date of the last day on which the present application could be submitted.

35 U.S.C. §156(c)(4), 37 C.F.R. §1.720(h): no other patent term has been extended for the same regulatory review period for the product

The regulatory review period for the Approved Product has not been the basis for extension of any other patent term.

Applicant claims patent term extension of **1826 days**, calculated under 37 C.F.R. §1.775(c)-(d) as follows:

(c) The length of the regulatory review period for the Approved Product, a human drug, is 4734 days, which is the sum of —

(1) The number of days in the period beginning on the date an exemption under subsection (i) of section 505 ... of the [FDCA] became effective for the approved product (IND effective date April 12, 2006) and ending on the date the application was initially submitted for such product under those sections (NDA submission date September 30, 2009) ...; - 1267 days - and

(2) The number of days in the period beginning on the date the application was initially submitted for the approved product under ... subsection (b) of section 505 ... of the [FDCA] (NDA submission date September 30, 2009) and ending on the date such application was approved under such section (March 29, 2019). - 3467 days

(d) The term of the patent as extended for a human drug ... will be determined by—

(1) Subtracting from the number of days determined by the Secretary of Health and Human Services to be in the regulatory review period: - 3244 days (4734 days above, less 1490 days below)

(i) The number of days in the periods of paragraphs (c)(1) and (c)(2) of this section which were on and before the date on which the patent issued (May 11, 2010); - 1490 days

(ii) The number of days in the periods of paragraphs (c)(1) and (c)(2) of this section during which it is determined under 35 U.S.C. 156(d)(2)(B) by the Secretary of Health and Human Services that applicant did not act with due diligence; - 0 days

(iii) One-half the number of days remaining in the period defined by paragraph (c)(1) of this section after that period is reduced in accordance with paragraphs (d)(1)(i) and (ii) of this section; half days will be ignored for purposes of subtraction; - 0 days

(2) By adding the number of days determined in paragraph (d)(1) of this section (3244 days) to the original term of the patent (October 16, 2026) as shortened by any terminal disclaimer; - September 3, 2035

(3) By adding 14 years to the date of approval of the application under ... subsection (b) of section 505 ... of the Federal Food, Drug, and Cosmetic Act (March 29, 2019); - March 29, 2033

(4) By comparing the dates for the ends of the periods obtained pursuant to paragraphs (d)(2) and (d)(3) of this section with each other and selecting the earlier date; - March 29, 2033

(5) Because the original patent was issued after September 24, 1984,

(i) By adding 5 years to the original expiration date of the patent or any earlier date set by terminal disclaimer; - October 16, 2031 - and

(ii) By comparing the dates obtained pursuant to paragraphs (d)(4) and (d)(5)(i) of this section with each other and selecting the earlier date - October 16, 2031 (1826 days after the expiration date)

* * *

37 C.F.R. §1.740(a)(13): A statement that applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services ... any information which is material to the determination of entitlement to the extension sought

Applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

37 C.F.R. §1.740(a)(14): The prescribed fee for receiving and acting upon the application for extension

Please charge the required fee of $1,120.00 pursuant to 37 C.F.R. §1.20(j) for receiving and acting upon this application to Deposit Account No. 601920. Please charge additional fee(s) or underpayment of fee(s) to Deposit Account No. 601920 under 37 CFR 1.16 and 1.17, and please credit any overpayment of fee(s) to Deposit Account No. 601920.

37 C.F.R. §1.740(a)(15): The name, address, and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed

Correspondence relating to the application for patent term extension should be addressed to:

Dr. Kirsten Grüneberg
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* * *

Certification under 37 C.F.R. §1.740(b)

The undersigned hereby certifies that the instant application, including exhibits and supporting papers, is being submitted as one original and two copies thereof, for a total of three copies, in accordance with 37 C.F.R. §1.740(b).

Conclusion

In accordance with the above statements and the exhibits provided herewith, Applicant respectfully requests the extension of the term of the '947 patent under 35 U.S.C. §156 due to regulatory delay.

Respectfully Submitted,
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CERTIFICATE OF MAILING

I hereby certify that this correspondence (along with any paper referred to as being attached or enclosed) and fee is being deposited with the United States Postal Service with sufficient postage as Priority Mail Express® with label number EJ 028 863 791 US in an envelope addressed to:

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

on May 24, 2019.

Signature

Dr. Kirsten Grueneberg
Reg. No. 47,297

LIST OF EXHIBITS

Exhibit	Contents
A	U.S. Patent No. 7,713,947 ("the '947 patent")
B	Certificate of Correction of June 15, 2010 in the '947 patent
C	Assignment from inventor DE LUCA to LABORATORIES SERONO SA
D	Assignment (Merger/Change of Name) from LABORATORIES SERONO SA to MERCK SERONO SA
E	Assignment from inventors YTHIER, MUNAFO, and LOPEZ-BRESNAHAN to MERCK SERONO SA
F	Letter from EMD Serono, Inc. of Billerica, MA, which is the Marketing Applicant for Mavenclad, authorizing Applicant Merck Serono SA to rely upon Marketing Applicant's activities
G	Power of Attorney submitted May 21, 2019
H	Mavenclad Label
I	P.2.2 Drug Product information, submitted in NDA 22561 in May 2018, pages 20-32
J	FDA Approval Letter for Mavenclad NDA 22561
K	Email with Timestamp sent 4:42 pm on March 29, 2019 from Sandra Folkendt (FDA) to Tammy Sarnelli (Marketing Applicant)
L	Leustatin (cladribine) NDA 020229: listing and label
M	Maintenance Fee Statement (First Maintenance Fee Payment)
N	Maintenance Fee Statement (Second Maintenance Fee Payment)
O	Letter from FDA showing date of IND application 74634
P	Letter signed October 13, 2009 from FDA to Marketing Applicant (receipt of NDA 22561)
Q	Letter signed August 22, 2011 from FDA to Marketing Applicant (receipt of withdrawal of NDA 22561)
R	Letter signed June 13, 2018 from FDA to Marketing Applicant (receipt of NDA 22561)
S	Chronology of Major Communications between FDA and Marketing Applicant (for IND application 74634 and NDA 22561)

EXHIBIT A

US007713947B2

(12) **United States Patent**
De Luca et al.

(10) **Patent No.: US 7,713,947 B2**
(45) **Date of Patent: May 11, 2010**

(54) **CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS**

(75) Inventors: **Giampiero De Luca**, Conches/Geneva (CH); **Arnaud Ythier**, Collex-Bossy (CH); **Alain Munafo**, Tartegnin (CH); **Maria Lopez-Bresnahan**, Lincoln, MA (US)

(73) Assignee: **Merck Serono S.A.**, Coinsins, Vaud (CH)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 300 days.

(21) Appl. No.: **11/722,018**

(22) PCT Filed: **Dec. 20, 2005**

(86) PCT No.: **PCT/EP2005/056954**

§ 371 (c)(1),
(2), (4) Date: **Jun. 18, 2007**

(87) PCT Pub. No.: **WO2006/067141**

PCT Pub. Date: **Jun. 29, 2006**

(65) **Prior Publication Data**

US 2009/0081163 A1 Mar. 26, 2009

Related U.S. Application Data

(60) Provisional application No. 60/638,669, filed on Dec. 22, 2004.

(30) **Foreign Application Priority Data**

Dec. 22, 2004 (EP) 04106909

(51) **Int. Cl.**

A61K 31/52	(2006.01)
A61K 31/7076	(2006.01)
A61K 38/21	(2006.01)
A61K 9/00	(2006.01)

(52) **U.S. Cl.** **514/46**; 424/85.6

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,964,848 A * 10/1990 Bloom 604/6.03
5,506,214 A * 4/1996 Beutler 514/46
2010/0021429 A1 * 1/2010 Brentzel et al. 424/85.6

FOREIGN PATENT DOCUMENTS

EP 0 626 853 B1 4/2000
WO WO 2004/087101 A2 10/2004

OTHER PUBLICATIONS

Beutler, E. et al. "Marrow Suppression Produced by Repeated Doses of Cladribine", *Acta Haematol*, 1994, pp. 10-15, vol. 91.
Beutler, E. et al. "Treatment of Multiple Sclerosis and Other Autoimmune Diseases With Cladribine", *Seminars in Hematology*, Jan. 1, 1996, pp. 45-52, vol. 33, No. 1, Supplement 1.
Beutler, E. et al. "The treatment of chronic progressive multiple sclerosis with cladribine", *Proc. Natl. Acad. Sci. USA*, Feb. 1996, pp. 1716-1720, vol. 93.
Ellison, G. et al. "Oral Cladribine for Multiple Sclerosis", *Neurology*, Mar. 1997, P03.070, pp. A174-A175, vol. 48, No. 3, XP008047069.
Grieb, P. et al. "Effect of Repeated Treatments with Cladribine (2-Chlorodeoxyadenosine) on Blood Counts in Multiple Sclerosis Patients", *Archivum Immunologiae et Therapiae Experimentalis*, 1995, pp. 323-327, vol. 43, No. 5-6.
Kazimierczuk, Z. et al. "Synthesis of 2'-Deoxytubercidin, 2'-Deoxyadenosine, and Related 2'-Deoxynucleosides via a Novel Direct Stereospecific Sodium Salt Glycosylation Procedure", *J. Am. Chem. Soc.*, 1984, pp. 6379-6382, vol. 106, No. 21.
Kurtzke, J. "Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS)", *Neurology*, Nov. 1983, pp. 1444-1452, vol. 33.
Langtry, H. et al. "Cladribine: A Review of its Use in Multiple Sclerosis", *Biodrugs*, May 1998, pp. 419-433, vol. 9, No. 3.
Lassmann, H. et al. "Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy", *TRENDS in Molecular Medicine*, Mar. 2001, pp. 115-121, vol. 7, No. 3.
Lublin, F. et al. "Defining the clinical course of multiple sclerosis: Results of an international survey", *Neurology*, Apr. 1996, pp. 907-911, vol. 46.
Lucchinetti, C. et al. "Multiple sclerosis: recent developments in neuropathology, pathogenesis, magnetic resonance imaging studies and treatment", *Current Opinion in Neurology*, 2001, pp. 259-269, vol. 14.

(Continued)

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(57) **ABSTRACT**

The present invention is related to the use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis, wherein the preparation is to be orally administered and wherein re-treatments are possible.

48 Claims, No Drawings

OTHER PUBLICATIONS

Mattson, D. "Update on the diagnosis of multiple sclerosis", *Expert Review of Neurotherapeutics*, May 2002, pp. 319-327, vol. 2, No. 3.
McDonald, W. et al. "Recommended Diagnostic Criteria for Multiple Sclerosis: Guidlines from the International Panel on the Diagnosis of Multiple Sclerosis", *Annals of Neurology*, Jul. 2001, pp. 121-127, vol. 50, No. 1.
Miller, R. et al. "Therapeutic advances in ALS", *Neurology*, 1996, pp. S217, vol. 47, Suppl. 4.
Noseworthy, J. et al. "Multiple Sclerosis", *The New England Journal of Medicine*, Sep. 28, 2000, pp. 938-952, vol. 343, No. 13.
Poser, C. et al. "New Diagnostic Criteria for Multiple Sclerosis: Guidelines for Research Protocols", *Annals of Neurology*, Mar. 1983, pp. 227-231, vol. 13, No. 3.
Rice, G. et al. "Cladribine and progressive MS: Clinical and MRI outcomes of a multicenter controlled trial", *Neurology*, Mar. 2000, pp. 1145-1155, vol. 54.
Romine, J. et al. "A Double-Blind, Placebo-Controlled, Randomized Trial of Cladribine in Relapsing-Remitting Multiple Sclerosis", *Proceedings of the Association of American Physicians*, Jan./Feb. 1999, pp. 35-44, vol. 111, No. 1.
Schumacher, G. et al. "Problems of Experimental Trials of Therapy in Multiple Sclerosis: Report by the Panel on the Evaluation of Experimental Trials of Therapy in Multiple Sclerosis", *Annals New York Academy of Sciences*, Mar. 31, 1965, pp. 552-568, vol. 122.
Selby, R. et al. "Safety and Tolerability of Subcutaneous Cladribine Therapy in Progressive Multiple Sclerosis", *Can. J. Neurol. Sci.*, 1998, pp. 295-299, vol. 25.
Sipe, J. et al. "A neurologic rating scale (NRS) for use in multiple sclerosis", *Neurology*, Oct. 1984, pp. 1368-1372, vol. 34.
Stelmasiak, Z. et al. "A pilot trial of cladribine (2-chlorodeoxyadenosine) in remitting-relapsing multiple sclerosis", *Med. Sci Monit.*, 1998, pp. 4-8, vol. 4, No. 1.

* cited by examiner

CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

CROSS-REFERENCE TO RELATED APPLICATION

This application is the U.S. national stage application of International Patent Application No. PCT/EP2005/056954, filed Dec. 20, 2005, which claims the benefit of U.S. Provisional Patent Application No. 60/638,669, filed Dec. 22, 2004, the disclosures of which are hereby incorporated by reference in their entireties, including all figures, tables and amino acid or nucleic acid sequences.

FIELD OF THE INVENTION

The present invention relates to the use of multiple doses of Cladribine for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis.

BACKGROUND OF THE INVENTION

Multiple sclerosis (MS) is the most known chronic inflammatory demyelinating disease of the central nervous system in humans. The onset of the disease typically occurs during ages 20 to 40. Women are affected approximately twice as often as men.

Over time, MS may result in the accumulation of various neurological disabilities. Clinical is disability in MS is presumed to be a result of repeated inflammatory injury with subsequent loss of myelin and axons, leading to tissue atrophy.

MS is manifested in physical symptoms (relapses and disability progression), Central Nervous System (CNS) inflammation, brain atrophy and cognitive impairment. Presenting symptoms include focal sensory deficits, focal weakness, visual problems, imbalance and fatigue. Sexual impairment and sphincter dysfunction may occur. Approximately half of the patients with MS may experience cognitive impairment or depression.

MS is now considered to be a multi-phasic disease and periods of clinical quiescence (remissions) occur between exacerbations. Remissions vary in length and may last several years but are infrequently permanent.

Four courses of the disease are individualized: relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR) multiple sclerosis.

More than 80% of patients with MS will initially display a RR course with clinical exacerbation of neurological symptoms, followed by a recovery that may or may not be complete (Lublin and Reingold, *Neurology,* 1996, 46:907-911).

During RRMS, accumulation of disability results from incomplete recovery from relapses. Approximately, half of the patients with RRMS switch to a progressive course, called SPMS, 10 years after the diseased onset. During the SP phase, worsening of disability results from the accumulation of residual symptoms after exarcerbation but also from insidious progression between exacerbations (Lublin and Reingold above). 10% of MS patients have PPMS which is characterized by insidious progression of the symptoms from the disease onset. Less than 5% of patients have PRMS and are often considered to have the same prognosis as PPMS. It is suggested that distinct pathogenic mechanisms may be involved in different patient sub-groups and have wide-ranging implications for disease classification (Lassmann et al., 2001, *Trends Mol. Med.,* 7, 115-121; Lucchinetti et al., *Curr. Opin. Neurol.,* 2001, 14, 259-269).

MS onset is defined by the occurrence of the first neurological symptoms of CNS dysfunction. Advances in cerebrospinal fluid (CSF) analysis and magnetic resonance imaging (MRI) have simplified the diagnostic process and facilitated early diagnostic (Noseworthy et al., *The New England Journal of Medicine,* 2000, 343, 13, 938-952). The International Panel on the Diagnosis of MS issued revised criteria facilitating the diagnosis of MS and including MRI together with clinical and para-clinical diagnostic methods (Mc Donald et al., 2001, *Ann. Neurol.,* 50:121-127).

Current medications for MS which are disease modifying treatments, i.e. modifying the course of MS, modulate or suppress the immune system. There are four FDA approved immunomodulating agents for RRMS: three beta interferons (Betaseron®, Berlex; Avonex®, Biogen; Rebif®, Serono) and Glatimarer Acetate (Copaxone®, Amgen). There is also one FDA approved immunosuppressing drug for worsening MS, Mitoxantrone (Novantrone®, Amgen). Several other immunosuppressive agents are used, although not FDA approved.

Among them, Cladribine, a chlorinated purine analogue 2-chloro-2'deoxyadenosine analogue (2-CdA), has been suggested to be useful in the treatment of MS (EP 626853B1 and U.S. Pat. No. 5,506,214).

Several clinical studies with Cladribine in patients with multiple sclerosis have investigated the use of i.v. and s.c. Cladribine in MS.

Two double-blind, placebo controlled Phase II studies were conducted respectively in the treatment of Chronic Progressive MS (Selby et al., 1998, *Can. J. Neurol. Sci.,* 25:295-299) and Relapsing-Remitting MS respectively (Romine et al., 1999, *Proceedings of the Association of American Physicians,* 111, 1, 35-44).

In the first trial, the Cladribine dose used was 0.1 mg/kg/day for 7 days by continuous i.v. infusion. The treatment for repeated for 4 consecutive months.

In the second clinical trial, the Cladribine dose used was 0.07 mg/kg/day for 5 days by subcutaneous injection. The treatment was repeated for 6 consecutive months.

In addition, placebo controlled Phase III study was conducted in patients with primary progressive (PP) or secondary progressive (SP) multiple sclerosis (Rice at al., 2000, *Neurology,* 54, 5, 1145-1155). In this study, both patient groups received Cladribine by subcutaneous injection at a dose of 0.07 mg/kg/day. The treatment was repeated for either 2 months or 6 months.

The Phase II clinical studies provided evidence for the positive effects of Cladribine in patients with MS in terms of Kutzke Extended Disability Status Scale (EDSS), Scripps Neurologic rating Scale (SNRS) scores and Magnetic Resonance Imaging (MRI) findings (Beutler et al., 1996, *Proc. Nat. Acad. Sci. USA,* 93, 1716-1720; Romine et al., 1999 above). Phase II study results, were positive on the significant reduction of MRI-measured brain lesions (Rice at al., 2000, above).

Some adverse effects (AEs), such as increased incidence of infections related to compromised immune function or myelosuppression, were observed with the highest doses (Selby et al., 1998, above; Beutler et al., 1994, *Acta hematol,* 91:10-15). Due to the narrow margin of safety between the efficacy dose and the dose of occurrence of AEs, to date, all clinical trials for Cladribine in multiple sclerosis have been conducted using either i.v. or s.c. administration. As a result, Beutler et al. (Beutler et al., 1996, *Semin. Hematol.*

33, 1(S1), 45-52) excluded the oral route for the treatment of multiple sclerosis with Cladribine.

Grieb et al. reported a small trial in 11 patients with remitting-relapsing multiple sclerosis (Grieb et al., 1995, *Archivum Immunologiae et Therapiae Experimentalis,* 43 (5-6), 323-327) wherein Cladribine has been orally administered during 6 monthly courses of 5 days at a total dose of about 4-5.7 mg/kg (patients of about 52 and about 75 kilos, respectively) i.e. a total effective dose of 2-2.85 mg/kg. For some patients, a single re-treatment of 5 days was performed at a cumulative dose of 0.4-0.66 mg/kg after a cladribine free-period of 3 or 6 months. The side effects observed with the regimen above were said to be less severe than the ones observed in the study on patients suffering from chronic progressive multiple sclerosis treated by i.v. infusion of Cladribine (Sipe et al., 1994, *Lancet,* 344, 9-13) but were still present. In addition, the therapeutic efficacy of the oral regimen above versus the i.v. infusion therapy was questioned (Grieb et al., 1995, above) and a group of "non-responders" has been identified (Stelmasiak et al., 1998, *Laboratory Investigations,* 4(1), 4-8).

Therefore, it would be desirable to have a method for treating multiple sclerosis comprising the oral administration of Cladribine that would permit the same or improved effect on MS lesions while decreasing the occurrence and/or severity adverse events. In addition, as MS is a chronic disease, it would be desirable to decrease the occurrence and/or severity adverse events in such a way that re-treatments are possible. A sustained benefit of Cladribine treatment between the treatment periods is also desirable.

SUMMARY OF THE INVENTION

The present invention is directed towards a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, wherein the preparation is to be the orally administered. Particularly, the invention is directed towards a use of Cladribine for the preparation of a medicament for the treatment of relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis and wherein re-treatments are possible.

An embodiment of the invention provides an improved dosing regimen for Cladribine in the treatment of multiple sclerosis.

An additional embodiment of the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein adverse effects are reduced, allowing further use of Cladribine.

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein the Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In another embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction treatment wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance treatment wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The "total dose" or "cumulative dose" refers to the total dose of Cladribine administered during the treatment, i.e. the dose reached at the end of the treatment that is calculated by adding the daily doses. For example, the total dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days is 3.5 mg/kg or the total dose of Cladribine corresponding to a treatment of 0.35 mg/kg Cladribine per day during 5 days is 1.7 mg/kg.

"The total effective dose" or "cumulative effective dose" refers to the bioavailable dose of Cladribine after a given administration period, i.e. the bioavailable dose reached at the end of the treatment that is calculated by adding the daily doses reduced by the bioavailability coefficient. For example, the total effective dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days wherein the bioavailability of Cladribine is of about 40% is 1.4 mg/kg or the total effective dose of Cladribine corresponding to a treatment of 0.35 mg/kg Cladribine per day during 5 days wherein the bioavailability of Cladribine is of about 40% is 0.7 mg/kg.

Typically, the bioavailability of Cladribine or of a Cladribine formulation used in the context of this invention is from about 30% to about 90%, preferably from about 40% to about 60%, such as about 50%.

"A week" refers to a period of time of or about 5, about 6 or about 7 days.

"A month" refers to a period of time of or about 28, about 29, about 30 or about 31 days.

"Treatment" comprises the sequential succession of an "induction treatment" and at least a "maintenance treatment". Typically, a treatment according to the invention comprises an "induction treatment" and about one or about two or about three maintenance treatments. Typically, a treatment according to the invention is of about 2 years (about 24 months) or about 3 years (about 36 months) or about 4 years (about 48 months).

An "Induction Treatment" consists in the sequential succession of (i) an induction period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention is orally administered and (ii) a Cladribine-free period. An induction period lasts up to about 4 months or up to about 3 month or up to about 2 months. For example, an induction period lasts for about 2 to about 4 months. An induction period consists in the oral administration of Cladribine or a pharmaceutical preparation thereof during about 1 to about 7 days each month.

A "Cladribine-free period" is a period wherein no Cladribine is administered to the patient. During a Cladribine-free period, the patient can be [illegible] administration of Cladribine.

dosed with a placebo-pill or another drug except. A Cladribine-free period lasts up to about 10 months or up to 9 months or up to about 8 months. For example, a Cladribine-free period lasts from about 8 to about 10 months, typically at least of about 8 months.

A "Maintenance Treatment" consists in the sequential succession of (i) a maintenance period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention is orally administered at a lower dose than the Cladribine dose orally administered during the induction treatment and (ii) a Cladribine-free period. A maintenance period lasts for up to about 4 months, or up to about 3 months, or up to about 2 months, preferably up to about 2 months. For example, a maintenance period lasts for about 2 to about 4 months, preferably for about 2 months. A maintenance period consists in the oral administration of Cladribine or of a pharmaceutical preparation thereof during about 1 to about 7 days each month.

Within the context of this invention, the beneficial effect, including but not limited to an attenuation, reduction, decrease or diminishing of the pathological development after onset of the disease, may be seen after one or more a "treatments", after an "induction treatment", after a "maintenance treatment" or during a Cladribine-free period.

"Daily dose" refers to the total dose of Cladribine orally administered to the patient each day of administration. The daily dose can be reached through a single or several administrations per day, such as for example once a day, twice a day or three times a day.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinetic properties, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

Patients suffering from MS can be defined for example as having clinically definite or laboratory-definite MS according to Schumacher or Poser criteria (Schumacher et al., 1965, *Ann. NY Acad. Sci.* 1965; 122:552-568; Poser et al., 1983, *Ann. Neurol.* 13(3): 227-31).

"Relapses" involve neurologic problems that occur over a short period, typically days but sometimes as short as hours or even minutes. These attacks most often involve motor, sensory, visual or coordination problems early in the disease. Later, bladder, bowel, sexual and cognitive problems may be shown. Sometimes the attack onset occurs over several weeks. Typical MS relapse involves a period of worsening, with development of neurological deficits, then a plateau, in which the patient is not getting any better but also not getting any worse followed by a recovery period. Recovery usually begins within a few weeks.

"Efficacy" of a treatment according to the invention can be measured based on changes in the course of disease in response to a use according to the invention. For example, treatment of MS efficacy can be measured by the frequency of relapses in RRMS and the presence or absence of new lesions in the CNS as detected using methods such as MRI technique (Miller et al., 1996, *Neurology,* 47(Suppl 4): S217; Evans et al., 1997, *Ann. Neurology,* 41:125-132).

The observation of the reduction and/or suppression of MRI T_1 gadolinium-enhanced lesions (thought to represent areas of active inflammation) gives a primary efficacy variable.

Secondary efficacy variables include MRI T_1 enhanced brain lesion volume, MRI T_1 enhanced lesion number, MRI T_2 lesion volume (thought to represent total disease burden, i.e. demyelination, gliosis, inflammation and axon loss), MRI T_1 enhanced hypointense lesion volume (thought to represent primarily demyelination and axon loss), time-to-progression of MS, frequency and severity of exacerbations and time-to-exacerbation, Expanded Disability Status Scale score and Scripps Neurologic Rating Scale (SNRS) score (Sipe et al., 1984, *Neurology,* 34, 1368-1372). Methods of early and accurate diagnosis of multiple sclerosis and of following the disease progression are described in Mattson, 2002, *Expert Rev. Neurotherapeutics,* 319-328.

Degree of disability of MS patients can be for example measured by Kurtzke Expanded Disability Status Scale (EDSS) score (Kurtzke, 1983, *Neurology,* 33, 1444-1452). Typically a decrease in EDSS score corresponds to an improvement in the disease and conversely, an increase in EDSS score corresponds to a worsening of the disease.

Cladribine (2-CdA)

2-CdA and its pharmacologically acceptable salts may be used in the practice of this invention.

Cladribine can be formulated in any pharmaceutical preparation suitable for oral administration. Representative oral formulations of 2-CdA are described in (WO 96/19230; WO 96/19229; U.S. Pat. Nos. 6,194,395; 5,506,214; WO 2004/087100; WO 2004/087101), the contents of which are incorporated herein by reference. Examples of ingredients for oral formulations are given below.

Processes for preparing 2-CdA are well known in the art. For example, the preparation of 2-CdA is described in (EP 173,059; WO 04/028462; WO 04/028462; U.S. Pat. No. 5,208,327; WO 00/64918) and Robins et al., *J. Am. Chem. Soc.,* 1984, 106: 6379. Alternatively, pharmaceutical preparations of 2-CdA may be purchased from Bedford Laboratories, Bedford, Ohio.

Oral administration of Cladribine may be in capsule, tablet, oral suspension, or syrup form. The tablet or capsules may contain from about 3 to 500 mg of Cladribine. Preferably they may contain about 3 to about 10 mg of Cladribine, more preferably about 3, about 5 or about 10 mg of Cladribine. The capsules may be gelatin capsules and may contain, in addition to Cladribine in the quantity indicated above, a small quantity, for example less than 5% by weight, magnesium stearate or other excipient. Tablets may contain the foregoing amount of the compound and a binder, which may be a gelatin solution, a starch paste in water, polyvinyl alcohol in water, etc. with a typical sugar coating.

Compositions

Compositions of this invention may further comprise one or more pharmaceutically acceptable additional ingredient(s) such as alum, stabilizers, antimicrobial agents, buffers, coloring agents, flavoring agents, adjuvants, and the like.

Compositions of this invention may be in the form of tablets or lozenges formulated in a conventional manner. For example, tablets and capsules for oral administration may contain conventional excipients including, but not limited to, binding agents, fillers, lubricants, disintegrants and wetting agents. Binding agents include, but are not limited to, syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and polyvinylpyrrolidone. Fillers include, but are not limited to, lactose, sugar, microcrystalline cellulose, maizestarch, calcium phosphate, and sorbitol. Lubricants include, but are not limited to, magnesium stearate, stearic acid, talc, polyethylene glycol, and silica. Disintegrants include, but are not limited to, potato starch and sodium starch glycollate. Wetting agents include, but are not limited to, sodium lauryl sulfate). Tablets may be coated according to methods well known in the art.

Compositions of this invention may also be liquid formulations including, but not limited to, aqueous or oily suspensions, solutions, emulsions, syrups, and elixirs. The compositions may also be formulated as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain additives including, but not limited to, suspending agents, emulsifying agents, non-aqueous vehicles and preservatives. Suspending agent include, but are not limited to, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents include, but are not limited to, lecithin, sorbitan monooleate, and acacia. Non-aqueous vehicles include, but are not limited to, edible oils, almond oil, fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol. Preservatives include, but are not limited to, methyl or propyl p-hydroxybenzoate and sorbic acid.

Combination

According to the invention, Cladribine can be administered alone or in combination with IFN-beta, prophylactically or therapeutically to an individual prior to, simultaneously or sequentially with other therapeutic regimens or agents (e.g. multiple drug regimens), in a therapeutically effective amount, especially therapeutic agents for the treatment of multiple sclerosis. Active agents that are administered simultaneously with other therapeutic agents can be administered in the same or different compositions and in the same or different routes of administration.

In one embodiment, when Cladribine is administered in combination with WFN-beta, WFN-beta is administered during the Cladribine-free period.

In another embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered after the "treatment" according to the invention.

The term "interferon-beta (IFN-β)", as used herein, is intended to include fibroblast interferon in particular of human origin, as obtained by isolation from biological fluids or as obtained by DNA recombinant techniques from prokaryotic or eukaryotic host cells, as well as its salts, functional derivatives, variants, analogs and active fragments.

IFN-β suitable in accordance with the present invention is commercially available e.g. as Rebif® (Serono), Avonex® (Biogen) or Betaferon® (Schering). The use of interferons of human origin is also preferred in accordance with the present invention. The term interferon, as used herein, is intended to encompass salts, functional derivatives, variants, analogs and active fragments thereof.

Rebif® (recombinant human interferon-β) is the latest development in interferon therapy for multiple sclerosis (MS) and represents a significant advance in treatment. Rebi® is interferon (IFN)-beta 1a, produced from mammalian cell lines. It was established that interferon beta-1a given subcutaneously three times per week is efficacious in the treatment of Relapsing-Remitting Multiple Sclerosis (RRMS). Interferon beta-1a can have a positive effect on the long-term course of MS by reducing number and severity of relapses and reducing the burden of the disease and disease activity as measured by MRI.

The dosing of IFN-β in the treatment of relapsing-remitting MS according to the invention depends on the type of IFN-β used.

In accordance with the present invention, where IFN is recombinant IFN-β1b produced in *E. Coli*, commercially available under the trademark Betaseron®, it may preferably be administered sub-cutaneously every second day at a dosage of about of 250 to 300 μg or 8 MIU to 9.6 MIU per person.

In accordance with the present invention, where IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Avonex®, it may preferably be administered intramuscularly once a week at a dosage of about of 30 μg to 33 μg or 6 MIU to 6.6 MIU per person.

In accordance with the present invention, when IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Rebif®, it may preferably be administered sub-cutaneously three times a week (TIW) at a dosage of 22 to 44 μg or 6 MIU to 12 MIU per person.

Patients

Patients according to the invention are patients suffering from multiple sclerosis, preferably RRMS or early SPMS.

In an embodiment of the invention, patients are selected from human males or females between 18 and 55 years age.

In another embodiment of the invention, patients had at least one relapse within the prior 12 months of the treatment.

Use According to the Invention

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months or up to about 3 months or up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months or up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (ii) period lasts up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (ii) period lasts at least about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period (ii) lasts up to about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (iv) period lasts up to about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (iv) period lasts at least about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free periods (ii) and/or (iv) last between about 8 and about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein a placebo-pill is administered during the Cladribine-free period.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period is free of any administration.

In another further embodiment, the invention provides a use according to the invention wherein the maintenance period lasts up to about 4 months, or up to about 3 months, or up to about 2 months, preferably up to about 2 months.

In another further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the maintenance period (iii) is about 1.7 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

In a preferred embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i)

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period (iii) lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In another embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period (iii) is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period (iii) lasts up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months; the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In a preferred embodiment, the invention provides Cladribine for use as a medicament for the treatment of multiple sclerosis wherein the medicament is to be orally administered following the sequential steps below:

(i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period (iii) lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine

reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine about 3 to 30 mg Cladribine, preferably 5 to 20 mg Cladribine, most preferably 10 mg Cladribine.

In another further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the total effective dose of Cladribine reached at the end of the induction period is about 1.4 mg/kg and the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered once a day during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered several times a day administered once a day during the induction period, preferably twice or three times a day, more preferably twice a day.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 1 to about 7 days per month, preferably from about 5 to about 7 days per month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 0.02 days/kg to about 0.08 days/kg per month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 0.02 days/kg to about 0.08 days/kg per month during the maintenance period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 2 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 3 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 4 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 5 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 6 each month during the induction period.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 4 each month during the induction period and wherein the pharmaceutical formulation is a pharmaceutical formulation described in WO 2004/087101 or in WO 2004/087100.

In another embodiment, the invention provides a use of Cladribine according to any of the preceding claims wherein the pharmaceutical formulation is to be administered in combination with interferon-beta.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.5 mg/kg to about 3.5 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical formulation thereof in a patient in need thereof comprising the following steps:

(i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;

(ii) A Cladribine-free period wherein no Cladribine is administered;

(iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);

(iv) A Cladribine-free period wherein no Cladribine is administered.

In another further embodiment, the invention provides a method according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

In a preferred embodiment, the invention provides a method of treating multiple sclerosis with Cladribine, wherein Cladribine is orally administered following the sequential steps below:

(i) Administering Cladribine, such that the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) Administering no Cladribine during a Cladribine free period;

(iii) Administering Cladribine such that the total dose of Cladribine reached at the end of a maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);

(iv) And optionally, a Cladribine-free period wherein no Cladribine is administered.

In a further preferred embodiment, the invention provides a method wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months.

In a further preferred embodiment, the invention provides a method wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the total effective dose of Cladribine reached at the end of the induction period is about 1.4 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months, or up to about 8 months.

In a further preferred embodiment, the invention provides a method wherein the maintenance period lasts up to about 4 months, or up to about 3 months or up to about 2 months.

In a further preferred embodiment, the invention provides a method wherein the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the maintenance period is followed by a Cladribine-free period.

In another further embodiment, the invention provides a method according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

In another further embodiment, the invention provides a method according to the invention wherein the total effective dose of Cladribine reached at the end of the induction period is about 1.4 mg/kg and the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be orally administered at a daily dose of about 3 to about 30 mg.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be orally administered at a daily dose of about 10 mg.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is orally administered about 1 to about 7 days per month during the induction period.

In another further embodiment, the invention provides a method according to the invention wherein the steps (iii) are repeated at least one or two times.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be administered in combination with interferon-beta.

EXAMPLES

The following abbreviations refer respectively to the definitions below:

kg (kilogram), µg (microgram), mg (milligram), AEs (Adverse effects), CNS (Cnetral nervous system), CSF (Cerebrospinal fluid), EDSS (Expanded Disability Status Scale, SNRS (Scripps Neurologic Rating Scale), IFN (interferon), i.v. (intra-veinous), MIU (Million International units), MS (multiple sclerosis), MRI (Magnetic resonance imaging), p.o. (per os), PPMS (Primary progressive multiple sclerosis), PRMS (Progressive relapsing multiple sclerosis), RRMS (Relapsing-remitting multiple sclerosis), SPMS (Secondary progressive multiple sclerosis), s.c. (subcutaneous), TIW (Three times a week), 2-CdA (2-chloro-2'deoxyadenosine or Cladribine), UI; (International unit).

The efficacy and safety of oral Cladribine administration, eventually multi-dose administration, according to the invention can be assessed for example following the protocol below:

Example 1

Oral Cladribine in the Treatment of Relapsing Forms of MS

A study of sixty patients with relapsing forms of clinically definite multiple sclerosis is undertaken. Each patient is first examined for normal hepatic, renal, and bone marrow functioning to establish baseline values.

Patients are selected from Male or Female, between 18 and 55 years of age who had one or more relapses within the prior 12 months. Female patients are non-pregnant female. Patients are randomly assigned to one of the treatment groups listed in Table 1 below:

TABLE 1

Group	2-CdA
1	—
2	1.75 mg/kg
3	3.5 mg/kg

Each of the patients in Groups 2 and 3 receives 3 mg or 10 mg 2-CdA (1, 2 or 3 administration(s) a day depending on the patient's weight) combined in cyclodextrin formulation as described in WO 2004/087101, Example 3. The Compositions of the Cladribine formulations in 3 mg or 10 mg 2-CdA tablets containing hydroxypropyl-beta-cyclodextrin are listed in Table 2 below:

TABLE 2

Name of ingredients	Formula mg/tablet	Formula mg/tablet
Cladribine-2-hydroxypropyl-β-cyclodextrin- complex*	153.75 equivalent to 10 mg 2-CdA	30.60 equivalent to 3 mg 2-CdA
Sorbitol powder	44.25	68.4
Magnesium Stearate (vegetable grade)	2.0	1.00
Total	200.0	100

*Cladribine is complexed and lyophilised with 2-hydroxypropyl-β-cyclodextrin as a separate process as described in WO 2004/087101.

Examples of administration schemes for the induction period depending on the patient's weight are given below in Tables 3 and 4 for the target doses of 1.75 mg/kg and 3.5 mg/kg respectively. For the maintenance period, the example of administration scheme of Table 3 is applicable.

TABLE 3

Patient weight ranges (kg)			Total target dose (kg) equivalent		Number of pills (10 mg)/induction period		
	Mid		to 1.75 mg/kg		Month	Month	
Min	range	Max	Min	Max	1	2	Total
40	42.5	44.9	28	31.4	4	3	7
45	47.5	49.9	31.5	34.9	4	4	8
50	52.5	54.9	35	38.4	5	4	9
55	57.5	59.9	38.5	41.9	5	5	10
60	62.5	64.9	42	45.4	5	5	10
65	67.5	69.9	45.5	48.9	6	5	11
70	72.5	74.9	49	52.4	6	6	12
75	77.5	79.9	52.5	55.9	7	6	13
80	82.5	84.9	56	59.4	7	6	13
85	87.5	89.9	59.5	62.9	7	7	14
90	92.5	94.9	63	66.4	8	7	15
95	97.5	99.9	66.5	69.9	8	8	16
100	102.5	104.9	70	73.4	9	8	17
105	107.5	109.9	73.5	76.9	9	9	18
110	112.5	114.9	77	80.4	9	9	18
115	117.5	119.9	80.5	83.9	10	9	19

TABLE 4

Patient weight ranges (kg)			Total target dose (kg) equivalent		Number of pills (10 mg)/induction period				
	Mid		to 3.5 mg/kg		Month	Month	Month	Month	
Min	range	Max	Min	Max	1	2	3	4	Total
40	42.5	44.9	56	62.9	4	4	3	3	14
45	47.5	49.9	63	69.9	4	4	4	4	16
50	52.5	54.9	70	76.9	5	4	4	4	17
55	57.5	59.9	77	83.9	5	5	5	4	19
60	62.5	64.9	84	90.9	6	5	5	5	21
65	67.5	69.9	91	97.9	6	6	5	5	22
70	72.5	74.9	98	104.9	6	6	6	6	24
75	77.5	79.9	105	111.9	7	7	6	6	26
80	82.5	84.9	112	118.9	7	7	7	6	27
85	87.5	89.9	119	125.9	7	7	7	7	28
90	92.5	94.9	126	132.9	8	8	7	7	30
95	97.5	99.9	133	139.9	8	8	8	8	32
100	102.5	104.9	140	146.9	9	8	8	8	33
105	107.5	109.9	147	153.9	9	9	9	8	35
110	112.5	114.9	154	160.9	10	9	9	9	37
115	117.5	119.9	161	167.9	10	10	9	9	38

In Group 1 patients receive a placebo (saline) for 4 months followed by 8 months of no treatment.

In Group 2 patients receive a daily oral administration of Cladribine for about 5 days a month during 2 months (induction period) of 2-CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg (total dose of about 1.75 mg/kg for a bioavailability of about 40%); followed by administration of placebo for 2 months; followed by 8 months of no treatment.

In Group 3 patients receive a daily oral administration of Cladribine for about 5 days a month during 4 months (induction period) of 2-CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 4 months approximates about 1.4 mg/kg (total dose of about 3.5 mg/kg for a bioavailability of about 40%); followed by 8 months of no treatment.

Beginning at month 13, all 3 patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 months of no treatment.

Finally, beginning at month 25, all patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 more months of no treatment.

Patients are monitored to determine whether there is any progression or improvement of brain lesions associated with progression of MS through MRI scans and neurological examination as described in Miller et al., 1996, above; Evans et al., 1997, above; Sipe et al., 1984, above; and Mattson, 2002, above. All patients have a baseline and MRI study (brain or spinal cord, according to localization of the lesions) at month 12.

The patient's disability progression and the time for having a first relapse are monitored as well as the proportion of relapse-fee patients at 24 months.

Lymphocyte markers and monocyte counts are monitored in the patients.

Patients in Groups 2 and 3 have a decrease in brain lesions.

The data show that the 2-CdA regimen consisting in the succession of an induction treatment and maintenance treatments is efficient in decreasing brain lesions and no severe adverse effect is observed.

The invention claimed is:

1. A method of treating multiple sclerosis comprising the oral administration of a formulation comprising cladribine, wherein the formulation is to be orally administered following the sequential steps below:

(i) an induction period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period of between about 8 and about 10 months wherein no cladribine formulation is administered;

(iii) a maintenance period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the maintenance period

is lower than the total dose of cladribine reached at the end of the induction period (i); and

(iv) a cladribine-free period wherein no cladribine formulation is administered.

2. The method according to claim 1, wherein the induction period lasts up to about 4 months.

3. The method according to claim 2, wherein the induction period lasts about 2 months.

4. The method according to claim 2, wherein the induction period lasts about 3 months.

5. The method according to claim 2, wherein the induction period lasts about 4 months.

6. The method according to claim 2, wherein the induction period lasts up to about 3 months.

7. The method according to claim 1, wherein the total dose of cladribine reached at the end of the induction period is about 1.7 mg/kg.

8. The method according to claim 1, wherein the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg.

9. The method according to claim 1, wherein the cladribine-free (iv) period lasts about 10 months.

10. The method according to claim 1, wherein the maintenance period lasts about 4 months.

11. The method according to claim 1, wherein the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

12. The method according to claim 1, wherein the formulation is to be orally administered following the sequential steps below:

(i) an induction period wherein said cladribine formulation is orally administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period of between about 8 and about 10 months wherein no cladribine formulation is administered;

(iii) a maintenance period wherein said cladribine formulation is administered and wherein the total dose of cladribine reached at the end of the maintenance period is lower than the total dose of cladribine reached at the end of the induction period (i); and

(iv) a cladribine-free period wherein no cladribine formulation is administered;

wherein the induction period lasts up to 4 months; the cladribine-free period (ii) lasts up to 10 months; the maintenance period (iii) lasts up to 2 months; the cladribine-free period (iv) lasts up to 10 months; the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated one, two or three times.

13. The method according to claim 12, wherein the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

14. The method according to claim 12, wherein the formulation is to be orally administered at a daily dose of 3 to 30 mg cladribine.

15. The method according to claim 14, wherein the formulation is to be orally administered at a daily dose of 10 mg cladribine.

16. The method according to claim 1, wherein the formulation is orally administered 1 to 7 days per month during the induction period.

17. The method according to claim 1, wherein the steps (iii) to (iv) are repeated at least one or two times.

18. The method according to claim 1, wherein said cladribine formulation is to be administered in combination with interferon-beta.

19. The method according to claim 12, wherein said cladribine formulation is to be administered in combination with interferon-beta.

20. A method of treating multiple sclerosis comprising the oral administration of a formulation comprising cladribine following the sequential steps below:

(i) an induction period lasting from about 2 months to about 4 months wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period lasting from about 8 months to about 10 months, wherein no cladribine is administered;

(iii) a maintenance period lasting from about 2 months to about 4 months, wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the maintenance period is lower than the total dose of cladribine reached at the end of the induction period (i);

(iv) a cladribine-free period wherein no cladribine is administered.

21. The method according to claim 20, wherein the induction period lasts about 4 months.

22. The method according to claim 20, wherein the induction period lasts about 2 months.

23. The method according to claim 20, wherein the total dose of cladribine reached at the end of the induction period is about 1.7 mg/kg.

24. The method according to claim 20, where the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg.

25. The method according to claim 20, wherein the cladribine-free period (ii) lasts about 10 months.

26. The method according to claim 20, wherein the cladribine-free (iv) period lasts 10 months.

27. The method according to claim 20, wherein the maintenance period lasts about 2 months.

28. The method according to claim 20, wherein the formulation is orally administered following the sequential steps below:

(i) an induction period wherein said formulation is administered orally and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period wherein no cladribine is administered;

(iii) a maintenance period wherein said formulation is administered orally and wherein the total dose of cladribine reached at the end of the maintenance period is lower than the total dose of cladribine reached at the end of the induction period (i); (iv) a cladribine-free period wherein no cladribine is administered;

wherein the maintenance period (iii) lasts about 2 months; the cladribine-free period (iv) lasts about 10 months; the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeatedly performed one, two or three times.

29. The method according to claim 20, wherein the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

30. The method according to claim 20, wherein the formulation is orally administered at a daily dose of 3 to 30 mg cladribine.

31. The method according to claim 20, wherein the formulation is orally administered at a daily dose of 10 mg cladribine.

32. The method according to claim 20, wherein the formulation is orally administered 1 to 7 days per month during the induction period.

33. The method according to claim 20, wherein the steps (iii) to (iv) are repeated at least one time.

34. The method according to claim 20, wherein the steps (iii) to (iv) are repeated at least two times.

35. The method according to claim 20, wherein the formulation is administered in combination with interferon-beta.

36. A method of treating multiple sclerosis comprising the oral administration of a formulation comprising cladribine following the sequential steps below:

(i) an induction period lasting from about 2 months to about 4 months wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

(ii) a cladribine-free period lasting from about 8 months to about 10 months, wherein no cladribine is administered;

(iii) a maintenance period lasting from about 2 months to about 4 months, wherein said formulation is orally administered and wherein the total dose of cladribine reached at the end of the maintenance period is about 1.7 mg/kg;

(iv) a cladribine-free period wherein no cladribine is administered.

37. The method according to claim 36, wherein the induction period lasts about 4 months.

38. The method according to claim 36, wherein the induction period lasts about 2 months.

39. The method according to claim 36, wherein the total dose of cladribine reached at the end of the induction period is about 1.7 mg/kg.

40. The method according to claim 36, where the total dose of cladribine reached at the end of the induction period is about 3.5 mg/kg.

41. The method according to claim 36, wherein the cladribine-free period (ii) lasts about 10 months.

42. The method according to claim 36, wherein the cladribine-free (iv) period lasts 10 months.

43. The method according to claim 36, wherein the maintenance period lasts about 2 months.

44. The method according to claim 36, wherein the formulation is orally administered at a daily dose of 3 to 30 mg cladribine.

45. The method according to claim 36, wherein the formulation is orally administered at a daily dose of 10 mg cladribine.

46. The method according to claim 36, wherein the formulation is orally administered 1 to 7 days per month during the induction period.

47. The method according to claim 36, wherein the steps (iii) to (iv) are repeated at least one or two times.

48. The method according to claim 36, wherein the formulation is administered in combination with interferon-beta.

* * * * *

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,713,947 B2
APPLICATION NO. : 11/722018
DATED : May 11, 2010
INVENTOR(S) : Giampiero De Luca et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1,
Line 31, "Clinical is disability" should read --Clinical disability--.

Column 7,
Line 32, "WFN-beta, WFN-beta" should read --IFN-beta, IFN-beta--.

Column 14,
Line 12, "UI; (International unit)" should read --UI (International unit)--.

Signed and Sealed this

Fifteenth Day of June, 2010

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

EXHIBIT C

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
GIAMPIERO DE LUCA	07/12/2007

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Property Type	Number
Application Number:	11722018

OP $40.00 11722018

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NAME OF SUBMITTER:	FRANK C. EISENSCHENK, PH.D.

Total Attachments: 2
source=executedassignment#page1.tif
source=executedassignment#page2.tif

1040 US

SER-125

ASSIGNMENT

WHEREAS, I, the undersigned, residing at the indicated address given below, have invented certain new and useful improvements in **CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS**, for which an application for United States Letters Patent was filed June 18, 2007, as Serial No. 11/722,018.

WHEREAS, LABORATOIRES SERONO S.A., a corporation of the country of ~~France~~ Switzerland, having a place of business at Zone Industrielle de l'Ouriettaz, CH-1170, Aubonne, Switzerland, is desirous of acquiring the entire right, title, and interest in and to said invention and in and to any Letters Patent which may be granted therefor in the United States and in any and all foreign countries;

12/07/2007

NOW, THEREFORE, in view of LABORATOIRES SERONO S.A.'s review and evaluation of our patent disclosure and other valuable consideration, receipt of which is hereby acknowledged, I, the undersigned, have sold, assigned, and transferred, and by these presents do sell, assign, and transfer, unto said LABORATOIRES SERONO S.A., its successors and assigns, the full and exclusive right to the said invention in the United States and its territorial possessions and in all foreign countries and the entire right, title, and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations, and extensions thereof.

I hereby authorize and request the Patent Office Officials in the United States and in any and all foreign countries to issue any and all of said Letters Patent, when granted, to LABORATOIRES SERONO S.A., as the assignees of the entire right, title, and interest in and to the same, for the sole use and behoof of LABORATOIRES SERONO S.A., its successors and assigns.

FURTHER, I agree that I will communicate to LABORATOIRES SERONO S.A., or its representatives, any facts known to me respecting said invention; testify in any legal proceedings; sign all lawful papers; execute all divisional, continuation, substitution, renewal, and reissue applications; execute all necessary assignment papers to cause any and all of said Letters Patent to be issued to LABORATOIRES SERONO S.A.; make all rightful oaths; and generally do everything possible to aid LABORATOIRES SERONO S.A., its successors and assigns, to obtain and enforce proper protection for said invention in the United States and in any and all foreign countries.

IN TESTIMONY WHEREOF, I have hereunto set my hand this 12/07 day of July, 2007.

Signed _____

Giampiero de Luca
Chemin des Conches 15B
CH-1231 Conches/Geneva
Switzerland

WITNESS:

Signature: _____

Printed Name: David RICOL

Date: July 12th, 2007

EXHIBIT D

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	CHANGE OF NAME

CONVEYING PARTY DATA

Name	Execution Date
LABORATOIRES SERONO SA	12/12/2008

RECEIVING PARTY DATA

Name:	MERCK SERONO SA
Street Address:	Centre Industriel
City:	Coinsins, Vaud
State/Country:	SWITZERLAND
Postal Code:	1267

PROPERTY NUMBERS Total: 51

Property Type	Number
Application Number:	10515032
Application Number:	10510015
Application Number:	10499100
Application Number:	10517726
Application Number:	10510014
Application Number:	10540234
Application Number:	10548364
Application Number:	10556417
Application Number:	10546843
Application Number:	10576509
Application Number:	12341490
Application Number:	10573369
Application Number:	10570122
Application Number:	10573625
Application Number:	10583218

OP $2040.00 10515032

Application Number:	11659174
Application Number:	11575415
Application Number:	11722533
Application Number:	11915913
Application Number:	12064287
Application Number:	11915476
Application Number:	11912432
Application Number:	11997181
Application Number:	11916097
Application Number:	12158572
Application Number:	12067221
Application Number:	12094905
Application Number:	12067224
Application Number:	12094869
Application Number:	12096110
Application Number:	12096125
Application Number:	12094921
Application Number:	12278831
Application Number:	12158539
Application Number:	12096107
Application Number:	12301249
Application Number:	11915508
Application Number:	11915521
Application Number:	12064284
Application Number:	10565763
Application Number:	11720560
Application Number:	11722033
Application Number:	11722527
Application Number:	11722018
Application Number:	11814389
Application Number:	10579105
Application Number:	10738123
Application Number:	11025834
Application Number:	12137898
Application Number:	12492387

Application Number:	12492371

CORRESPONDENCE DATA

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Address Line 4: GAINESVILLE, FLORIDA 32614-2950

ATTORNEY DOCKET NUMBER:	SER.MISC/SLS
NAME OF SUBMITTER:	FRANK C. EISENSCHENK, PH.D.

Total Attachments: 21

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Béatrice EHLERS
NOTARY

ARTICLES OF ASSOCIATION

of

Merck Serono SA

a public limited company with registered office in Coinsins

*****

12 December 2008

*****

MERCK SERONO SA

ARTICLES OF ASSOCIATION

TITLE I
NAME - REGISTERED OFFICE
PURPOSE - DURATION

Article 1: Name

Under the name MERCK SERONO SA, a public limited liability company governed by these articles of association and by Title XXVI of the Swiss Code of Obligations (hereinafter also referred to as CO), is hereby constituted.

Article 2: Registered office

The registered office of the company is at Coinsins (Vaud Canton).

Article 3: Purpose

The purpose of the company is:
(1) the conduct of the business of a holding company (acquiring and administering participations both in Switzerland and abroad) in the pharmaceutical and allied fields,
(2) research, development, creation, manufacture, consultancy, commercial sale and utilisation of technologies for the life sciences,
(3) registration and utilisation of patents,
(4) synthesis and commercial sale of biological products for therapeutic purposes,
(5) conclusion of partnership agreements, mergers and acquisitions of companies in the same business areas. The company may also effect all financial, commercial, industrial and real estate transactions and conclude all contracts appropriate to the development of its purpose or having a direct or indirect bearing upon such purpose.

Article 4: Duration

The company is incorporated for an indefinite duration.

TITLE II
SHARE CAPITAL
SHARES

Article 5: Share capital

The share capital is set at the sum of CHF 383,758,575 (three hundred and eighty-three million seven hundred and fifty-eight thousand five hundred and seventy-five francs), divided into

a) 11,013,040 registered "A" shares with limited transferability with a nominal value of CHF 10 (ten francs) each, fully paid up, and
b) 10,945,127 "B" bearer shares with a nominal value of CHF 25 (twenty-five francs) each, fully paid up.

Article 5 bis: Conditional capital

A. Conditional capital for option and/or convertible loans

The share capital of the company shall be increased by CHF 36,300,000 (thirty-six million three hundred thousand francs) at most, by the issue of 1,452,000 (one million four hundred and fifty-two thousand) "B" type bearer shares with a nominal value of CHF 25 (twenty-five) francs each to be paid up in full by the exercise of option and/or conversion rights granted in relation to the loans issued by member companies of the Serono Group.

The amount and conditions of the loans, together with the procedures and conditions for the exercise of option and/or conversion rights and the issue price are to be determined by the Board of Directors. The holders of convertible bonds or option rights carried by option bonds are entitled to acquire new shares.

The Board of Directors may issue loans which are directly underwritten by a consortium and subsequently placed with the public, subject to the following provisions.

The Board of Directors determines the conditions for the exercise of the preferential subscription right. Preferential subscription rights which have not been exercised

revert to the company. The Board of Directors may place them on market terms or allow them to expire.
The Board of Directors may cancel the shareholders' preferential subscription right if loans are issued to finance the acquisition of participations or other rights in companies or to finance research and development projects. If the Board of Directors abolishes the shareholders' preferential subscription right, the following provisions shall apply: (a) conversion rights may be exercised only for a maximum period of 15 years and option rights for 7 years from the date of issue of the related loan; (b) convertible and/or option loans must be issued on the standard market conditions (including the standard market conditions relating to protection of option and/or conversion right holders against dilution), and (c) the conversion and/or option price must correspond at least to the average of the prices paid on the Zurich stock market for shares in the company during the 5 day period prior to the determination of the definitive issue conditions for the convertible or option loan concerned.

B. Conditional capital for a stock option plan

The company's share capital shall be increased by CHF 14,452,550 (fourteen million four hundred and fifty-two thousand five hundred and fifty francs) at most, i.e. 578,102 (five hundred and seventy-eight thousand one hundred and two) "B" type bearer shares with a nominal value of CHF 25 (twenty-five francs) each, to be paid up in full by the exercise of option rights which the Board of Directors intends to grant to the staff of the member companies of the Serono Group and to the directors of the company.

The shareholders' subscription right does not apply to these new shares.

The Board of Directors shall stipulate in a regulation the conditions and procedures for granting options and for their exercise.

The shares may be subscribed at a price which is lower than the stock market price.

Article 5 ter: Authorised capital

Until 25 April 2008 the Board of Directors is authorised to increase the share capital by a maximum of CHF 190,471,500 (one hundred and ninety million four hundred and seventy-one thousand five hundred francs) by issuing a maximum of 7,618,860 (seven million six hundred and eighteen thousand eight hundred and sixty) "B" type bearer shares with a nominal value of CHF 25 (twenty-five francs) each, fully paid up. The Board of Directors may arrange for the share capital to be increased in its entirety or by tranches. Preferential subscription rights which have been granted

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but not exercised shall be placed at the disposal of the Board of Directors which shall use them in the interests of the company.

The Board of Directors is authorised to exclude the preferential subscription right of the shareholders in favour of a bank or another institution which directly underwrites the shares chosen by the Board of Directors if the bank or institution which underwrites the shares undertakes to offer the shareholders a right to subscribe to the newly issued shares in proportion to their current participation. The Board of Directors is likewise authorised to exclude the shareholders' preferential subscription right and to assign the shares or the preferential right to subscribe to shares to third parties in the event of the acquisition of a company or parts of a company, the taking of a participation in a business or a company, or similar transactions and the financing of such transactions.

The share issue price, the way in which payment for the shares is to be made and the date from which the new shares shall give an entitlement to dividends and the conditions for the exercise of the preferential subscription right shall be determined by the Board of Directors.

Article 6: Shares

6.1 Shares are of the registered or bearer type. They are numbered and signed by a director whose signature may be printed.

Each share gives entitlement to a proportional part of the profit and of the proceeds of liquidation.

6.2 In lieu of single registered or bearer shares, the company may issue certificates representing more than one share.

6.3 **Registered shares**
Registered shares are indivisible in relation to the company which acknowledges only one holder of each registered share. Shareholders who own registered shares must notify any change of address to the company. Any communication by the company to the shareholders is deemed to be valid when it is notified to the last recorded address of the shareholder.

6.4 **Register of registered shares**
The Board of Directors keeps a register of registered shares which indicates the name and address of the owners and beneficiaries from usufruct in registered shares.

Only the persons whose names appear on this register are regarded as being the owners of, or beneficiaries of usufruct in, registered shares in relation to the company.

No entry in the share register shall be made between the day on which the General Meeting is convened and the day following the date of the General Meeting.

6.5 **Transfer of registered shares**

The transfer of registered shares must be approved by the company. The Board of Directors has authority to decide. The request for authorisation must include a statement by which the purchaser of the shares certifies that he is taking the shares over in his own name and for his own account. The company will inform the applicant whether the transfer has been authorised or declined.

Entry in the register will be declined if the applicant has not specifically stated that he is purchasing the shares in his own name and for his own account.

Entry in the register may be declined for justified reasons associated with the registered purpose or the economic independence of the company and, in particular, when the purchaser is a person who competes with the company or a company or business in which it holds participations.

The company may, without stating reasons, withhold its approval for a share transfer by offering to take over the shares from the seller for its own account, for the account of other shareholders or for that of third parties at the real value at the time when the application for transfer is received by the company.

In the event of transfer by inheritance, the company must enter in the share register the name of the acquiring party, save where there is a justified reason for not doing so within the meaning of paragraph 3 above. In that assumption, if the company proposes to refuse the transfer, it must offer to take the shares over for its own account, for the account of other shareholders or for that of third parties at the real value at the time when the application for entry in the register is received by the company.

If the company proposes to purchase shares for the account of shareholders, it must respect the principle of equal treatment of the holders of registered shares.

After hearing the persons concerned, the Board of Directors may cancel with retroactive effect, the entries in the share register made on the basis of inaccurate declarations.

The above provisions likewise apply to the creation of usufruct in registered shares of the company.

Registered shares cannot be the subject of a pledge, guarantee or charge of any nature whatsoever without the specific prior approval of the Board of Directors which is free to decide whether to state reasons for its decision.

Reservations concerning the free transfer of registered shares will be indicated on the documents which represent the shares.

The provisions of Article 6.5 may be amended solely by a decision taken by a majority as stipulated in article 704, para 1, CO.

6.6 **Conversion of shares**

The General Meeting may decide at any time to convert all or some of the registered shares into bearer shares and vice versa.

Article 7: Increase of the share capital

7.1 The General Meeting may at any time decide to increase the share capital by issuing new registered or bearer shares. Each series of shares may itself be the subject of a specific issue.

7.2 Every shareholder is entitled to the proportion of newly issued shares corresponding to his previous participation. Where an increase in the share capital comprises an increase of registered shares and bearer shares in the same proportion, each holder of registered shares is entitled to subscribe to the registered shares only in proportion to the number of his registered shares and each shareholder who owns bearer shares is only entitled to subscribe to new bearer shares in proportion to the number of his shares.

TITLE III
ORGANISATION OF THE COMPANY

A. General Meeting

Article 8

8.1 The General Meeting is the supreme body of the company. Its decisions are binding on all of the shareholders.

8.2 Decisions of the General Meeting which are in breach of the law or articles of association may be contested by the Board of Directors and by each shareholder under the conditions stipulated in article 706 of the Code of Obligations.

Article 9

The General Meeting of shareholders has the inalienable right to:

9.1 adopt and amend the articles of association

9.2 appoint and dismiss members of the Board of Directors and the auditors

9.3 approve the annual report and financial statements of the group

9.4 approve the annual financial statements and determine the appropriation of the profit and, in particular, set the dividend.

9.5 grant a release to the members of the Board of Directors

9.6 take all decisions which are reserved for it by the law and by the articles of association.

Article 10

10.1 No decision may be taken upon matters which have not been placed on the agenda, save on the proposal to convene an Extraordinary General Meeting or to establish a special audit.

10.2 There is no need for an advance announcement of proposals which fall within the framework of items placed on the agenda or of deliberations which do not have to be followed by a vote.

Article 11

11.1 The General Meeting is held at the registered office of the company or at the place designated by the Board of Directors.

11.2 The Ordinary General Meeting is held each year within six month of the end of the financial year.

11.3 Extraordinary General Meetings are convened as often as is necessary, in particular in the cases stipulated by law and by decision of the General Meeting itself.

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Article 12

The General Meeting is convened by the Board of Directors and, if necessary, by the auditors. The liquidators are likewise entitled to convene a meeting.

Article 13

13.1 The General Meeting is convened not less than twenty days before the date set for it to be held by registered letter sent to each of the registered shareholders at the address stated in the register of registered shares and by publication in the Feuille Officielle Suisse du Commerce (Swiss Official Commercial Gazette).

13.2 The invitation to attend the General Meeting must indicate the items placed on the agenda, together with the motions of the Board of Directors and of the shareholders who have asked for the meeting to be convened or for an item to be placed on its agenda, provided that this has been notified in writing to the Secretariat of the Board of Directors not less than 45 days before the date set for the meeting. The invitation to attend must also state the date, place and time of the meeting.

13.3 Proposals for amendments to the articles of association shall be placed at the disposal of shareholders at the registered office of the company; an indication that they are so available must be given in the invitation to attend the meeting.

13.4 Invitations to attend the Ordinary General Meeting must inform the shareholders that the annual report, the profit and loss account together with the balance sheet and financial statements of the group and the auditor's report are available for consultation by the shareholders at the registered office of the company not less than twenty days before the General Meeting.

Article 14

Owners or representatives of all the shares may, if there is no opposition, hold a General Meeting without observing the formalities stipulated for it to be convened. As long as they are all present, this meeting is entitled to deliberate and act validly on all the matters which fall within the terms of reference of the General Meeting.

Article 15

Each share gives the entitlement to one vote.

Article 16

Each registered shareholder may arrange for all or some of his shares to be represented by a different person who must carry a written proxy.

Article 17

17.1 As a general rule, the General Meeting is validly constituted regardless of the number of shares which are represented.

17.2 Save where otherwise stipulated in the law or articles of association, the General Meeting takes its decisions and holds its elections by an absolute majority of the votes carried by the shares which are represented.

17.3 A decision of the General Meeting which receives not less than two-thirds of the votes carried by the shares which are represented and an absolute majority of the nominal values represented is required to change the registered purpose, introduce shares with privileged voting rights, restrict the transferability of registered shares, proceed to an authorised or conditional increase of the share capital, increase the share capital by means of equity, against a contribution in kind or with a view to the acquisition of assets and the granting of special advantages, limitation or cancellation of the preferential subscription right, transfer of the registered office of the company and winding up of the company without liquidation.

Article 18

18.1 Minutes of the General Meeting are written and must indicate the number, type, nominal value and category of the shares represented by the shareholders, the official bodies and the independent representatives and custodial representatives, the decisions and outcome of the elections, the requests for information and the answers given, together with statements which the shareholders ask to be recorded. The minutes are to be signed by the Chairman and by the Secretary of the meeting.

18.2 The extracts of the minutes which are issued must be certified true copies by a director or by any other person designated for this purpose.

Article 19

19.1 The General Meeting is chaired by the Chairman of the Board of Directors or by some other director designated by the Board of Directors. Failing this, a chairman of the day shall be appointed by the General Meeting.

19.2 The Chairman of the General Meeting appoints the secretary and the teller or tellers.

B. BOARD OF DIRECTORS

Article 20

20.1 The Board of Directors of the company comprises one or more members.

20.2 (Deleted)

Article 21

21.1 The Directors are appointed for a one-year term of office.

21.2 They may be re-elected indefinitely.

21.3 The Board of Directors shall appoint its Chairman and Secretary by a simple majority.

Article 22

22.1 The Board of Directors is convened by the Chairman or, on his instructions, by the Secretary as often as business so requires.

22.2 Minutes of its deliberations and decisions shall be written and signed by the Chairman and Secretary.

Article 23

23.1 Decisions of the Board of Directors are taken by a majority of the members present provided, however, that they constitute a majority of the Board of Directors.

23.2 In the event of a tied vote, the Chairman shall have a casting vote.

23.3 The decisions of the Board of Directors may also be taken in the form of approval given in writing to a proposal by a majority of all the directors who must all be informed of the proposal, unless a discussion is requested by any one of the members. These decisions must be recorded in the minutes.

Article 24

The Board of Directors has the most extensive possible powers to manage the company. It is authorised to take decisions on all matters which are not assigned to, or reserved for, the General Meeting and other bodies of the company.

Article 25

25.1 The Board may entrust all or part of the management and representation of the company to one or more directors (delegates) or to third parties who need not necessarily be shareholders.

25.2 It appoints procuration holders and other authorised representatives of the company.

25.3 It grants the right to sign individually or jointly on behalf of the company.

25.4 (Deleted)

Article 26

The Board of Directors shall adopt organisational rules.

C. EXECUTIVE COMMITTEE

(Deleted)

D. AUDIT BODY

Article 27

The General Meeting appoints the audit body to serve for one financial year if an ordinary or restricted audit must be performed.

It may decide not to elect an audit body, if:

1. the company is not subject to an ordinary audit
2. all of the shareholders agree to this, and

3. the company workforce does not exceed 10 full-time posts on an annual average.

This waiver shall likewise apply to subsequent years.

However, each shareholder is entitled to request a restricted audit no later than ten days before the General Meeting. A General Meeting (extraordinary) must then elect the audit body.

The statutory provisions apply to the tasks of the audit body.

In the event of an ordinary audit, the audit body must be present at the General Meeting. This may dispense with the presence of the audit body by a decision taken unanimously.

Article 27 bis

One or more natural persons or corporate bodies, together with partnerships, may act as the audit body.

The audit body must have its domicile, registered office or a branch entered in the register of commerce in Switzerland. When the company has more than one audit body, one at least must meet this requirement.

When the company is required to submit its annual financial statements for ordinary verification by an audit body in virtue of Art. 727 paras. 1, 2 or 3 and 727 para. 2 CO, the General Meeting shall elect an expert auditor approved within the meaning of the federal law on supervision of auditors of 16 December 2005 to act as the audit body.

When the company is required to submit its annual financial statements to a restricted verification by an audit body, the General Meeting shall elect an approved auditor within the meaning of the federal law on the supervision of auditors of 16 December 2005 to serve as the audit body. The right to dispense with the election of an audit body is reserved. The audit body must be independent within the meaning of Art. 728 or 729 CO.

The mandate of the audit body ends upon the approval of the latest annual financial statements. Its term of office may be renewed. The General Meeting may at any time dismiss the audit body with immediate effect.

TITLE IV
ANNUAL FINANCIAL STATEMENTS AND APPROPRIATION OF THE PROFIT

Article 28

The financial year begins on the first of January and ends on the thirty-first of December.

Article 29

29.1 The annual financial statements are drawn up in compliance with the provisions of Articles 662 to 670 CO.

29.2 The financial statements are drawn up as of on the thirty-first of December.

Article 30

30.1 Each year, one-twentieth of the profit for the financial year shall be set aside to a general reserve until the latter reaches one-fifth of the share capital which has already been paid up. Further amounts shall be set aside if any part of the reserve is used up.

30.2 The balance of the profit shall be appropriated in compliance with the decisions of the General Meeting, after consulting the Board of Directors.

30.3 The binding provisions of law concerning statutory reserves must be respected.

Article 31

The dividend shall be paid at the time fixed by the Board of Directors. Any dividend which has not been claimed within five years of the date on which it falls due shall be automatically time-barred in favour of the company.

TITLE V
LIQUIDATION

Article 32

32.1 When a decision to wind up the company is taken, the liquidation shall be effected by the Board of Directors, save where otherwise decided by the General Meeting.

32.2 At least one of the liquidators must be domiciled in Switzerland and have authority to represent the company.

32.3 The liquidators shall agree among themselves upon the method of signing on behalf of the company.

Article 33

33.1 In the course of liquidation, the powers of the bodies of the company are restricted to the actions required for this operation and which, by their nature, do not fall within the province of the liquidators.

33.2 The General Meeting of shareholders retains the right to approve the liquidation accounts and to grant a release for them.

33.3 The liquidator or liquidators cannot transfer to third parties against payment or against any other consideration, the assets and liabilities of the company which has been wound up, save in virtue of a decision taken by the General Meeting.

TITLE VI
PUBLICATIONS

Article 34

Publications for the company shall be made in the Feuille Officielle Suisse du Commerce (Swiss Official Commercial Gazette).

ARTICLES OF ASSOCIATION ADOPTED by the constituent General Meeting of 20 May 1987 and amended at the Extraordinary General Meeting of 12 December 2008.

Certified:

Beatrice EHLERS
NOTARY

Signed

Attestation

We hereby certify that, to the best of our knowledge, this is a correct translation of the respective document.

Bern, 08.06.2009

Inter-Translations SA, Bern

Inter-Translations SA
Pavillonweg 14 · CH-3001 Bern

Danielle Cesarov-Zaugg

SA
Bern

Béatrice EHLERS
NOTARY

MINUTES

of the Extraordinary General Meeting of Shareholders of

Laboratoires Serono SA

changed to

Merck Serono SA

a limited liability company with registered office in Coinsins

******

Minute No. 337 dated 12 December 2008

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Minute No. 337

RECORD OF PROCEEDINGS

IN THE YEAR TWO THOUSAND AND EIGHT, on Friday twelve December, at 10 am, I, the undersigned, BEATRICE EHLERS, notary in Lausanne for the Canton of Vaud, acting at the request of the Board of Directors, drew up the following authentic record of the proceedings of the Extraordinary General Meeting of Shareholders of

Laboratoires Serono SA

a limited liability company with registered office in Coinsins.

The meeting was chaired by Maître Markus Funk, domiciled in Chêne-Bougeries.

The undersigned notary drafted the record of proceedings which was drawn up in the authentic form required by law.

The Chairman noted the fact that the entire share capital amounting to 11,013,040 (eleven million thirteen thousand and forty) registered shares with a nominal value of CHF 10 (ten francs) each and 10,945,127 (ten million nine hundred and forty-five thousand one hundred and twenty-seven) bearer shares of CHF 25 (twenty-five francs) was represented, as stated in the attendance register which was produced to remain enclosed in the file of the company at the office of the undersigned notary; the meeting was therefore able to hold valid deliberations pursuant to the provisions of Article 701 of the Swiss Code of Obligations.

The agenda proposed by the Chairman and adopted unanimously was as follows:

1. Change of name – amendment to the articles of association
2. Other changes to the articles of association
3. Other business

1. Change of name – amendment to the articles of association

The Chairman explained the reasons for which it had been felt appropriate by the Board to recommend a change of name to the meeting; he proposed that the meeting should agree to adopt "Merck Serono SA" as the official name of the company in future.

The meeting unanimously agreed to the proposed name for the company.

On the basis of the decision which had thus been taken, the Chairman proposed that Article 1 of the articles of association be amended to now read as follows:

"Article 1: Name
Under the name Merck Serono SA, a public limited liability company governed by these articles of association and by title XXVI of the Swiss Code of Obligations (hereinafter also referred to as CO) is hereby constituted".

The new wording of article 1 was adopted unanimously.

2. Other changes to the articles of association
The Chairman explained to the meeting that the Board of Directors had felt it appropriate to use the present General Meeting as an opportunity to update the articles of association following the entry into force of the new law on companies on 1 January 2008. He therefore proposed the amendments set out below to the following articles:

The new wording of Article 9, para. 1, would be as follows:

"The General Meeting of shareholders has the inalienable right:
[...]"

Article 20, section 20.1, would now read as follows:

"The Board of Directors of the company comprises one or more members".

Article 27 is cancelled and replaced by Articles 27 and 27 bis to read as follows:

"Article 27
The General Meeting appoints the audit body for a term of one financial year if an ordinary or restricted audit has to be performed.

It may refrain from electing an audit body if:

1. *The company is not required to undergo an ordinary audit*
2. *The totality of the shareholders agree to this, and*
3. *The staff complement of the company does not exceed 10 full-time posts on an annual average.*

This waiver likewise applies to subsequent years.

However, each shareholder is entitled to require a restricted audit to take place no later than ten days before the General Meeting. An (extraordinary) General Meeting must then elect the audit body.

The tasks of the audit body shall be determined by the statutory provisions.

In the event of an ordinary audit, the audit body must attend the General Meeting. The latter may waive the requirement for the audit body to be present by a unanimous decision.

Article 27 bis
One or more natural persons or corporate bodies may be elected to act as the audit body, as may partnerships.
The domicile, registered office or a branch establishment of the audit body must be entered in the register of commerce in Switzerland. If the company has more than one audit body, at least one of them must satisfy this requirement.
If the company is required to submit its annual account statements for an ordinary audit by an audit body in virtue of Art 727, para. 1, ch. 2 or ch. 3 and 727, para. 2 CO, the General Meeting shall elect an approved expert auditor within the meaning of the federal law on the supervision of auditors of 16 December 2005 to act as the audit body.

Where the company is required to submit its annual account statements for a restricted audit by an audit body, the General Meeting shall elect an approved auditor within the meaning of the federal law on the supervision of auditors of 16 December 2005 to act as the audit body. The waiver of the election of an audit body is reserved.

The audit body must be independent within the meaning of Art 728 or 729 CO.
The mandate of the audit body ends with the approval of the final annual account statements. Its term of office may be renewed. The General Meeting may dismiss the audit body with immediate effect at any time".

The above amendments to the articles of association were all adopted unanimously.

3. Other business
The meeting gave full authority to the undersigned notary to arrange for the present record to be entered in the register of commerce.

There being no other items on the agenda and nobody else wishing to speak, the meeting was closed after these minutes had been read out and approved, ending with the signing by the Chairman and the notary in the year, month and on the day indicated above in Lausanne, at ten fifteen am.

The minute is signed: M. Funk – B. Ehlers, not.

SECOND CERTIFIED TRUE COPY
Delivered to the company,

Certified by:

(signature) B Ehlers
(Seal of Béatrice Ehlers NOTARY)

Attestation

We hereby certify that, to the best of our knowledge, this is a correct translation of the respective document.

Bern, 08.07.2009

Inter-Translations SA, Bern

Inter-Translations SA
Pavillonweg 14 • CH-3001 Bern

Danielle Cesarov-Zaugg

EXHIBIT E

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
ARNAUD YTHIER	07/15/2009
ALAIN MUNAFO	07/07/2009
MARIA LOPEZ-BRESNAHAN	03/11/2010

RECEIVING PARTY DATA

Name:	MERCK SERONO SA
Street Address:	CENTRE INDUSTRIEL
City:	COINSINS, VAUD
State/Country:	SWITZERLAND
Postal Code:	1267

PROPERTY NUMBERS Total: 1

Property Type	Number
Application Number:	11722018

OP $40.00 11722018

CORRESPONDENCE DATA

Fax Number: (352)372-5800
Correspondence will be sent via US Mail when the fax attempt is unsuccessful.
Phone: 3523758100
Email: FCE@SLSPATENTS.COM
Correspondent Name: FRANK C. EISENSCHENK, PH.D.
Address Line 1: P.O. BOX 142950
Address Line 4: GAINESVILLE, FLORIDA 32614-2950

ATTORNEY DOCKET NUMBER:	SER.125
NAME OF SUBMITTER:	FRANK C. EISENSCHENK, PH.D.

Total Attachments: 4
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source=executed-Asgn#page2.tif

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source=executed-Asgn#page4.tif

ASSIGNMENT

WHEREAS, we, the undersigned, residing at the indicated addresses given below, have invented certain new and useful improvements in **CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS**, for which an application for United States Letters Patent was filed June 18, 2007, as Serial No. 11/722,018.

WHEREAS, MERCK SERONO S.A., a corporation of the country of Switzerland, having a place of business at Centre Industriel, 1267 Coinsins, Vaud, Switzerland, is desirous of acquiring the entire right, title, and interest in and to said invention and in and to any Letters Patent which may be granted therefor in the United States and in any and all foreign countries;

NOW, THEREFORE, in view of MERCK SERONO S.A.'s review and evaluation of our patent disclosure and other valuable consideration, receipt of which is hereby acknowledged, I, the undersigned, have sold, assigned, and transferred, and by these presents do sell, assign, and transfer, unto said MERCK SERONO S.A., its successors and assigns, the full and exclusive right to the said invention in the United States and its territorial possessions and in all foreign countries and the entire right, title, and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations, and extensions thereof.

We hereby authorize and request the Patent Office Officials in the United States and in any and all foreign countries to issue any and all of said Letters Patent, when granted, to MERCK SERONO S.A., as the assignees of the entire right, title, and interest in and to the same, for the sole use and behoof of MERCK SERONO S.A., its successors and assigns.

FURTHER, we agree that we will communicate to MERCK SERONO S.A., or its representatives, any facts known to us respecting said invention; testify in any legal proceedings; sign all lawful papers; execute all divisional, continuation, substitution, renewal, and reissue applications; execute all necessary assignment papers to cause any and all of said Letters Patent to be issued to MERCK SERONO S.A.; make all rightful oaths; and generally do everything possible to aid MERCK SERONO S.A., its successors and assigns, to obtain and enforce proper protection for said invention in the United States and in any and all foreign countries.

IN TESTIMONY WHEREOF, I have hereunto set my hand this 15th day of July, 2009.

Signed _____

Arnaud Ythier
Route de Vireloup 88
1239 Collex-Bossy
Switzerland

WITNESS:

Signature: _____

Printed Name: CAROL JASCI

Date: 15.7.2009

IN TESTIMONY WHEREOF, I have hereunto set my hand this 07 day of July, 2009.

Signed _____

Alain Munafo
Rue des Pressoirs 6
1180 Tartegnin
Switzerland

WITNESS:

Signature: _____

Printed Name: CECE FORSMANN

Date: July 7th, 2009

IN TESTIMONY WHEREOF, I have hereunto set my hand this 11th day of March, 2010.

Signed _____

Maria Lopez-Bresnahan
145 South Great Road
Lincoln, MA 01773

WITNESS:

Signature: _____

Printed Name: Victoria Bresnahan

Date: 11- March - 2010

EXHIBIT F

EMD SERONO

Mary C. Till
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner for Patent Examination Policy
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

May 21, 2019

Re: Patent Term Extension for U.S. Patent No. 7,713,947

Dear Ms. Till:

This will acknowledge the approval by the FDA of New Drug Application (NDA) No. 022561 for Mavenclad (cladribine). NDA No. 022561 was submitted by EMD Serono, Inc.

On behalf of EMD Serono, Inc., Marketing Applicant for NDA No. 022561 for Mavenclad (cladribine), its predecessors, and affiliates, EMD Serono, Inc. hereby authorizes the patent owner of record, Merck Serono SA, in connection with this application for extension of the patent term of U.S. Patent No. 7,713,947, to rely upon the activities of EMD Serono, Inc., its predecessors, and affiliates, undertaken in connection with seeking approval by the Food and Drug Administration of NDA No. 022561. EMD Serono, Inc. and Merck Serono SA are both subsidiaries of Merck KGaA.

Respectfully submitted,

Signature: 

Name: Michael MacDougall
Title: Senior Vice President, General Counsel & Secretary
Company: EMD Serono, Inc.

EXHIBIT G

Copy as filed May 21, 2019

TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

This form is to be submitted with the Power of Attorney by Applicant Form to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form.

Application Number	11/722,018
Patent Number	7,713,947
Filing Date	June 18, 2007
Issue Date	May 11, 2010
First Named Inventor	Giampiero De Luca
Art Unit	1649
Examiner Name	BALLARD, KIMBERLY
Attorney Docket Number	000758US
Title	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

Signature of Applicant or Patent Practitioner

Signature	/Kirsten Grueneberg/
Name	Dr. Kirsten Grueneberg
Reg. No.	47,297
Customer No.	151167

Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

*Total of __1__ form(s) is/are submitted.

Copy as filed May 21, 2019

PTO/SB/81A (12-08)
Approved for use through 03/31/2021. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995 no persons are required to respond to a collection of information unless it displays a valid OMB control number

PATENT - POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Patent Number	7,713,947
Issue Date	May 11, 2010
First Named Inventor	Giampiero De Luca
Title	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
Attorney Docket No.	000758US

I hereby revoke all previous powers of attorney given in the above-identified patent.

☐ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the Customer Number identified in the box at right as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

151167

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified patent to:

☒ The address associated with the above-identified Customer Number.

OR

☐ The address associated with the Customer Number identified in the box at right:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☐ Inventor, having ownership of the patent.

OR

☒ Patent owner.

Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____.

SIGNATURE of Inventor or Patent Owner

Signature	Prisca Ballmoos	Date	May 21, 2019
Name	Prisca VON BALLMOOS / Willem DE WEERD	Telephone	
Title and Company	Authorized Representative Merck Serono S.A. / Authorized Representative Merck Serono S.A.		

NOTE: Signatures of all the inventors or patent owners of the entire interest or their representative(s) are required. If more than one signature is required, submit multiple forms, check the box below, and identify the total number of forms submitted in the blank below.

☒ A total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.31, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public, which is to update (and by the USPTO to process) the file of a patent or reexamination proceeding. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 15 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Copy as filed May 21, 2019

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Merck Serono S.A.

Application No./Patent No.: 7,713,947 Filed/Issue Date: May 11, 2010

Titled: CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

Merck Serono S.A., a corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.

states that it is:

1. ☒ the assignee of the entire right, title, and interest in;

2. ☐ an assignee of less than the entire right, title, and interest in (The extent (by percentage) of its ownership interest is _____ %); or

3. ☐ the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)

the patent application/patent identified above, by virtue of either:

A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel_____, Frame_____, or a copy* is attached.

OR

B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Giampiero DE LUCA To: LABORATOIRES SERONO S.A.

The document was recorded in the United States Patent and Trademark Office at Reel 019685, Frame 0061, or a copy* is attached.

2. From: LABORATOIRES SERONO SA To: MERCK SERONO SA

The document was recorded in the United States Patent and Trademark Office at Reel 023601, Frame 0156, or a copy* is attached.

3. From: Arnaud YTHIER; Alain MUNAFO; and Maria LOPEZ-BRESNAHAN To: MERCK SERONO S.A.

The document was recorded in the United States Patent and Trademark Office at Reel 024080, Frame 0041, or a copy* is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ *As required by 37 CFR 3.73(b)(1)(i), if a copy/copies is/are attached, the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Kirsten Grueneberg/ May 21, 2019

Signature Date

Dr. Kirsten Grueneberg 47,297

Printed or Typed Name Title or Registration Number

EXHIBIT H

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use MAVENCLAD safely and effectively. See full prescribing information for MAVENCLAD.

MAVENCLAD® (cladribine) tablets, for oral use
Initial U.S. Approval: 1993

WARNING: MALIGNANCIES and RISK OF TERATOGENICITY
See full prescribing information for complete boxed warning.

- **Malignancies**

MAVENCLAD may increase the risk of malignancy. MAVENCLAD is contraindicated in patients with current malignancy; evaluate the benefits and risks on an individual basis for patients with prior or increased risk of malignancy. (5.1)

- **Risk of Teratogenicity**

MAVENCLAD is contraindicated for use in pregnant women and in women and men of reproductive potential who do not plan to use effective contraception because of the risk of fetal harm. (5.2)

----------INDICATIONS AND USAGE----------

MAVENCLAD is a purine antimetabolite indicated for the treatment of relapsing forms of multiple sclerosis (MS), to include relapsing-remitting disease and active secondary progressive disease, in adults. Because of its safety profile, use of MAVENCLAD is generally recommended for patients who have had an inadequate response to, or are unable to tolerate, an alternate drug indicated for the treatment of MS *[see Warnings and Precautions (5)]*. (1)

Limitations of Use

MAVENCLAD is not recommended for use in patients with clinically isolated syndrome (CIS) because of its safety profile *[see Warnings and Precautions (5)]*. (1)

----------DOSAGE AND ADMINISTRATION----------

- Assessments are required prior to starting each MAVENCLAD treatment course. (2.1)
- Cumulative dosage of 3.5 mg/kg administered orally and divided into 2 treatment courses (1.75 mg/kg per treatment course). Each treatment course is divided into 2 treatment cycles. (2.2)
- MAVENCLAD is a cytotoxic drug. (2.4)
- Separate administration from any other oral drug by at least 3 hours. (2.4)

----------DOSAGE FORMS AND STRENGTHS----------

Tablets: 10 mg (3)

----------CONTRAINDICATIONS----------

- Patients with current malignancy. (4)
- Pregnant women, and women and men of reproductive potential who do not plan to use effective contraception during MAVENCLAD dosing and for 6 months after the last dose in each treatment course. (4, 8.3)
- HIV infection. (4)
- Active chronic infections (e.g., hepatitis or tuberculosis). (4)
- History of hypersensitivity to cladribine. (4, 5.8)
- Women intending to breastfeed on a MAVENCLAD treatment day and for 10 days after the last dose. (4, 8.2)

----------WARNINGS AND PRECAUTIONS----------

- Lymphopenia: Monitor lymphocyte counts before, during and after treatment. (5.3)
- Infections: Screen patients for latent infections; consider delaying treatment until infection is fully controlled. Vaccinate patients antibody-negative to varicella zoster virus prior to treatment. Administer anti-herpes prophylaxis in patients with lymphocyte counts less than 200 cells per microliter. Monitor for infections. (5.4)
- Hematologic toxicity: Measure complete blood count annually if clinically indicated after treatment. (5.5)
- Graft-versus-host-disease with blood transfusion: Irradiation of cellular blood components is recommended. (5.6)
- Liver injury: Obtain tests prior to treatment. Discontinue if clinically significant injury is suspected. (5.7)

----------ADVERSE REACTIONS----------

Most common adverse reactions (incidence > 20%) are upper respiratory tract infection, headache, and lymphopenia. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact EMD Serono at 1-800-283-8088 ext. 5563 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------DRUG INTERACTIONS----------

- Immunosuppressive drugs: Consider overlapping effects on immune system, when used sequentially. Concomitant use not recommended. (7.1)
- Hematotoxic drugs: Monitor patients for additive effects on the hematological profile. (7.3)
- Antiviral and antiretroviral drugs: Avoid concomitant use. (7.4)
- BCRP or ENT/CNT inhibitors: May alter bioavailability of cladribine. Avoid concomitant use. (7.5)

See 17 for PATIENT COUNSELING INFORMATION **and Medication Guide.**

Revised: 3/2019

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

WARNING: MALIGNANCIES AND RISK OF TERATOGENICITY

- **Malignancies**

Treatment with MAVENCLAD may increase the risk of malignancy. MAVENCLAD is contraindicated in patients with current malignancy. In patients with prior malignancy or with increased risk of malignancy, evaluate the benefits and risks of the use of MAVENCLAD on an individual patient basis. Follow standard cancer screening guidelines in patients treated with MAVENCLAD *[see Contraindications (4) and Warnings and Precautions (5.1)]*.

- **Risk of Teratogenicity**

MAVENCLAD is contraindicated for use in pregnant women and in women and men of reproductive potential who do not plan to use effective contraception because of the potential for fetal harm. Malformations and embryolethality occurred in animals. Exclude pregnancy before the start of treatment with MAVENCLAD in females of reproductive potential. Advise females and males of reproductive potential to use effective contraception during MAVENCLAD dosing and for 6 months after the last dose in each treatment course. Stop MAVENCLAD if the patient becomes pregnant *[see Contraindications (4), Warnings and Precautions (5.2), and Use in Specific Populations (8.1, 8.3)]*.

1 INDICATIONS AND USAGE

MAVENCLAD is indicated for the treatment of relapsing forms of multiple sclerosis (MS), to include relapsing-remitting disease and active secondary progressive disease, in adults. Because of its safety profile, use of MAVENCLAD is generally recommended for patients who have had an inadequate response to, or are unable to tolerate, an alternate drug indicated for the treatment of MS *[see Warnings and Precautions (5)]*.

Limitations of Use

MAVENCLAD is not recommended for use in patients with clinically isolated syndrome (CIS) because of its safety profile *[see Warnings and Precautions (5)]*.

2 DOSAGE AND ADMINISTRATION

2.1 Assessments Prior to Starting Each MAVENCLAD Treatment Course

Cancer Screening

Follow standard cancer screening guidelines because of the risk of malignancies *[see Boxed Warning and Warnings and Precautions (5.1)]*.

Pregnancy

Exclude pregnancy prior to treatment with MAVENCLAD in females of reproductive potential *[see Contraindications (4), Warnings and Precautions (5.2), and Use in Specific Populations (8.1, 8.3)]*.

Complete Blood Count (CBC)

Obtain a CBC with differential including lymphocyte count *[see Dosage and Administration (2.5) and Warnings and Precautions (5.3)]*. Lymphocytes must be:

- within normal limits before initiating the first treatment course
- at least 800 cells per microliter before initiating the second treatment course

If necessary, delay the second treatment course for up to 6 months to allow for recovery of lymphocytes to at least 800 cells per microliter. If this recovery takes more than 6 months, the patient should not receive further treatment with MAVENCLAD.

Infections *[see Warnings and Precautions (5.4)]*

- Exclude HIV infection.
- Perform tuberculosis screening.
- Screen for hepatitis B and C.
- Evaluate for acute infection. Consider a delay in MAVENCLAD treatment until any acute infection is fully controlled.
- Vaccination of patients who are antibody-negative for varicella zoster virus is recommended prior to initiation of MAVENCLAD.
- Administer all immunizations according to immunization guidelines prior to starting MAVENCLAD. Administer live-attenuated or live vaccines at least 4 to 6 weeks prior to starting MAVENCLAD.
- Obtain a baseline (within 3 months) magnetic resonance imaging prior to the first treatment course because of the risk of progressive multifocal leukoencephalopathy (PML).

Liver Injury

Obtain serum aminotransferase, alkaline phosphatase, and total bilirubin levels *[see Warnings and Precautions (5.7)]*.

2.2 Recommended Dosage

The recommended cumulative dosage of MAVENCLAD is 3.5 mg per kg body weight administered orally and divided into 2 yearly treatment courses (1.75 mg per kg per treatment course) (see Table 1). Each treatment course is divided into 2 treatment cycles:

Administration of First Treatment Course

- First Course/First Cycle: start any time.
- First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle.

Administration of Second Treatment Course

- Second Course/First Cycle: administer at least 43 weeks after the last dose of First Course/Second Cycle.
- Second Course/Second Cycle: administer 23 to 27 days after the last dose of Second Course/First Cycle.

Table 1 Dose of MAVENCLAD per Cycle by Patient Weight in Each Treatment Course

Weight Range	**Dose in mg (Number of 10 mg Tablets) per Cycle**	
kg	First Cycle	Second Cycle
40* to less than 50	40 mg (4 tablets)	40 mg (4 tablets)
50 to less than 60	50 mg (5 tablets)	50 mg (5 tablets)
60 to less than 70	60 mg (6 tablets)	60 mg (6 tablets)
70 to less than 80	70 mg (7 tablets)	70 mg (7 tablets)
80 to less than 90	80 mg (8 tablets)	70 mg (7 tablets)
90 to less than 100	90 mg (9 tablets)	80 mg (8 tablets)
100 to less than 110	100 mg (10 tablets)	90 mg (9 tablets)
110 and above	100 mg (10 tablets)	100 mg (10 tablets)

*The use of MAVENCLAD in patients weighing less than 40 kg has not been investigated.

Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days *[see How Supplied/Storage and Handling (16.1)]*. Do not administer more than 2 tablets daily.

Following the administration of 2 treatment courses, do not administer additional MAVENCLAD treatment during the next 2 years. Treatment during these 2 years may further increase the risk of malignancy *[see Warnings and Precautions (5.1)]*. The safety and efficacy of reinitiating MAVENCLAD more than 2 years after completing 2 treatment courses has not been studied.

2.3 Missed Dose

If a dose is missed, patients should not take double or extra doses.

If a dose is not taken on the scheduled day, then the patient must take the missed dose on the following day and extend the number of days in that treatment cycle. If two consecutive doses are missed, the treatment cycle is extended by 2 days.

2.4 Administration

MAVENCLAD tablets are taken orally, with water, and swallowed whole without chewing. MAVENCLAD can be taken with or without food.

Separate administration of MAVENCLAD and any other oral drugs by at least 3 hours during the 4 to 5 day MAVENCLAD treatment cycles *[see Clinical Pharmacology (12.6)]*.

MAVENCLAD is a cytotoxic drug. Follow applicable special handling and disposal procedures *[see References (15)]*. MAVENCLAD is an uncoated tablet and must be swallowed immediately once removed from the blister. If a tablet is left on a surface, or if a broken or fragmented tablet is released from the blister, the area must be thoroughly washed with water.

The patient's hands must be dry when handling the tablets and washed thoroughly afterwards. Avoid prolonged contact with skin.

2.5 Laboratory Testing and Monitoring to Assess Safety

Cancer Screening

Follow standard cancer screening guidelines in patients treated with MAVENCLAD *[see Dosage and Administration (2.1) and Warnings and Precautions (5.1)]*.

Complete Blood Count

Obtain complete blood count (CBC) with differential including lymphocyte count:

- before initiating the first treatment course of MAVENCLAD
- before initiating the second treatment course of MAVENCLAD
- 2 and 6 months after start of treatment in each treatment course; if the lymphocyte count at month 2 is below 200 cells per microliter, monitor monthly until month 6. *See Warnings and Precautions (5.3, 5.4)* for instructions based on the patient's lymphocyte counts and clinical status (e.g., infections). Hold MAVENCLAD therapy if the lymphocyte count is below 200 cells per microliter
- periodically thereafter and when clinically indicated *[see Warnings and Precautions (5.5)]*

2.6 Recommended Concomitant Medication

Herpes Prophylaxis

Administer anti-herpes prophylaxis in patients with lymphocyte counts less than 200 cells per microliter *[see Warnings and Precautions (5.4)]*.

3 DOSAGE FORMS AND STRENGTHS

MAVENCLAD is available as 10 mg tablets. The tablets are uncoated, white, round, biconvex, and engraved with a "C" on one side and "10" on the other side.

4 CONTRAINDICATIONS

MAVENCLAD is contraindicated:

- in patients with current malignancy *[see Warnings and Precautions (5.1)]*.
- in pregnant women and in women and men of reproductive potential who do not plan to use effective contraception during MAVENCLAD dosing and for 6 months after the last dose in each treatment course. May cause fetal harm *[see Warnings and Precautions (5.2) and Use in Specific Populations (8.1, 8.3)]*.
- in patients infected with the human immunodeficiency virus (HIV) *[see Warnings and Precautions (5.4)]*.
- in patients with active chronic infections (e.g., hepatitis or tuberculosis) *[see Warnings and Precautions (5.4)]*.
- in patients with a history of hypersensitivity to cladribine *[see Warnings and Precautions (5.8)]*.

- in women intending to breastfeed on a MAVENCLAD treatment day and for 10 days after the last dose *[see Use in Specific Populations (8.2)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Malignancies

Treatment with MAVENCLAD may increase the risk of malignancy. In controlled and extension clinical studies worldwide, malignancies occurred more frequently in MAVENCLAD-treated patients [10 events in 3,754 patient-years (0.27 events per 100 patient-years)], compared to placebo patients [3 events in 2,275 patient-years (0.13 events per 100 patient-years)]. Malignancy cases in MAVENCLAD patients included metastatic pancreatic carcinoma, malignant melanoma (2 cases), ovarian cancer, compared to malignancy cases in placebo patients, all of which were curable by surgical resection [basal cell carcinoma, cervical carcinoma in situ (2 cases)]. The incidence of malignancies in United States MAVENCLAD clinical study patients was higher than the rest of the world [4 events in 189 patient-years (2.21 events per 100 patient-years) compared to 0 events in United States placebo patients]; however, the United States results were based on a limited amount of patient data.

After the completion of 2 treatment courses, do not administer additional MAVENCLAD treatment during the next 2 years *[see Dosage and Administration (2.2)]*. In clinical studies, patients who received additional MAVENCLAD treatment within 2 years after the first 2 treatment courses had an increased incidence of malignancy [7 events in 790 patient-years (0.91 events per 100 patient-years) calculated from the start of cladribine treatment in Year 3]. The risk of malignancy with reinitiating MAVENCLAD more than 2 years after the completion of 2 treatment courses has not been studied.

MAVENCLAD is contraindicated in patients with current malignancy. In patients with prior malignancy or with increased risk of malignancy, evaluate the benefits and risks of the use of MAVENCLAD on an individual patient basis. Follow standard cancer screening guidelines in patients treated with MAVENCLAD.

5.2 Risk of Teratogenicity

MAVENCLAD may cause fetal harm when administered to pregnant women. Malformations and embryolethality occurred in animals *[see Use in Specific Populations (8.1)]*. Advise women of the potential risk to a fetus during MAVENCLAD dosing and for 6 months after the last dose in each treatment course.

In females of reproductive potential, pregnancy should be excluded before initiation of each treatment course of MAVENCLAD and prevented by the use of effective contraception during MAVENCLAD dosing and for at least 6 months after the last dose of each treatment course. Women who become pregnant during treatment with MAVENCLAD should discontinue treatment *[see Use in Specific Populations (8.1, 8.3)]*. MAVENCLAD is contraindicated for use in pregnant women and in women and men of reproductive potential who do not plan to use effective contraception.

5.3 Lymphopenia

MAVENCLAD causes a dose-dependent reduction in lymphocyte count. In clinical studies, 87% of MAVENCLAD-treated patients experienced lymphopenia. The lowest absolute lymphocyte counts occurred approximately 2 to 3 months after the start of each treatment course and were lower with each additional treatment course. In patients treated with a cumulative dose of MAVENCLAD 3.5 mg per kg over 2 courses as monotherapy, 26% and 1% had nadir absolute lymphocyte counts less than 500 and less than 200 cells per microliter, respectively. At the end of the second treatment course, 2% of clinical study patients had lymphocyte counts less than 500 cells per microliter; median time to recovery to at least 800 cells per microliter was approximately 28 weeks.

Additive hematological adverse reactions may be expected if MAVENCLAD is administered prior to or concomitantly with other drugs that affect the hematological profile *[see Drug Interactions (7.3)]*. The incidence of lymphopenia less than 500 cells per microliter was higher in patients who had used drugs to treat relapsing forms of MS prior to study entry (32.1%), compared to those with no prior use of these drugs (23.8%).

Obtain complete blood count (CBC) with differential including lymphocyte count prior to, during, and after treatment with MAVENCLAD. *See Dosage and Administration (2.1, 2.5) and Warnings and Precautions (5.4)* for timing of CBC measurements and additional instructions based on the patient's lymphocyte counts and clinical status (e.g., infections).

5.4 Infections

MAVENCLAD can reduce the body's immune defense and may increase the likelihood of infections. Infections occurred in 49% of MAVENCLAD-treated patients compared to 44% of placebo patients in clinical studies. The most frequent serious infections in MAVENCLAD-treated patients included herpes zoster and pyelonephritis *(see Herpes Virus Infections)*. Fungal infections were observed, including cases of coccidioidomycosis.

HIV infection, active tuberculosis, and active hepatitis must be excluded before initiation of each treatment course of MAVENCLAD *[see Contraindications (4)]*.

Consider a delay in initiation of MAVENCLAD in patients with an acute infection until the infection is fully controlled.

Initiation of MAVENCLAD in patients currently receiving immunosuppressive or myelosuppressive therapy is not recommended *[see Drug Interactions (7.1)]*. Concomitant use of MAVENCLAD with these therapies could increase the risk of immunosuppression.

Tuberculosis

Three of 1,976 (0.2%) cladribine-treated patients in the clinical program developed tuberculosis. All three cases occurred in regions where tuberculosis is endemic. One case of tuberculosis was fatal, and two cases resolved with treatment.

Perform tuberculosis screening prior to initiation of the first and second treatment course of MAVENCLAD. Latent tuberculosis infections may be activated with use of MAVENCLAD. In patients with tuberculosis infection, delay initiation of MAVENCLAD until the infection has been adequately treated.

Hepatitis

One clinical study patient died from fulminant hepatitis B infection. Perform screening for hepatitis B and C prior to initiation of the first and second treatment course of MAVENCLAD. Latent hepatitis infections may be activated with use of MAVENCLAD. Patients who are carriers of hepatitis B or C virus may be at risk of irreversible liver damage caused by virus reactivation. In patients with hepatitis infection, delay initiation of MAVENCLAD until the infection has been adequately treated.

Herpes Virus Infections

In controlled clinical studies, 6% of MAVENCLAD-treated patients developed a herpes viral infection compared to 2% of placebo patients. The most frequent types of herpes viral infections were herpes zoster infections (2.0% vs. 0.2%) and oral herpes (2.6% vs. 1.2%). Serious herpes zoster infections occurred in 0.2% of MAVENCLAD-treated patients.

Vaccination of patients who are antibody-negative for varicella zoster virus is recommended prior to initiation of MAVENCLAD. Administer live-attenuated or live vaccines at least 4 to 6 weeks prior to starting MAVENCLAD.

The incidence of herpes zoster was higher during the period of absolute lymphocyte count less than 500 cells per microliter, compared to the time when the patients were not experiencing this degree of lymphopenia. Administer anti-herpes prophylaxis in patients with lymphocyte counts less than 200 cells per microliter.

Patients with lymphocyte counts below 500 cells per microliter should be monitored for signs and symptoms suggestive of infections, including herpes infections. If such signs and symptoms occur, initiate treatment as clinically indicated. Consider interruption or delay of MAVENCLAD until resolution of the infection.

Progressive Multifocal Leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is an opportunistic viral infection of the brain caused by the JC virus (JCV) that typically only occurs in patients who are immunocompromised, and that usually leads to death or severe disability. Typical symptoms associated with PML are diverse, progress over days to weeks, and include progressive weakness on one side of the body or clumsiness of limbs, disturbance of vision, and changes in thinking, memory, and orientation leading to confusion and personality changes.

No case of PML has been reported in clinical studies of cladribine in patients with multiple sclerosis. In patients treated with parenteral cladribine for oncologic indications, cases of PML have been reported in the postmarketing setting.

Obtain a baseline (within 3 months) magnetic resonance imaging (MRI) before initiating the first treatment course of MAVENCLAD. At the first sign or symptom suggestive of PML, withhold MAVENCLAD and perform an appropriate diagnostic evaluation. MRI findings may be apparent before clinical signs or symptoms.

Vaccinations

Administer all immunizations according to immunization guidelines prior to starting MAVENCLAD. Administer live-attenuated or live vaccines at least 4 to 6 weeks prior to starting MAVENCLAD, because of a risk of active vaccine infection *(see Herpes Virus Infections)*. Avoid vaccination with live-attenuated or live vaccines during and after MAVENCLAD treatment while the patient's white blood cell counts are not within normal limits.

5.5 Hematologic Toxicity

In addition to lymphopenia *[see Warnings and Precautions (5.3)]*, decreases in other blood cells and hematological parameters have been reported with MAVENCLAD in clinical studies. Mild to moderate decreases in neutrophil counts (cell count between 1,000 cells per microliter and < lower limit of normal (LLN)) were observed in 27% of MAVENCLAD-treated patients, compared to 13% of placebo patients whereas severe decreases in neutrophil counts (cell count below 1,000 cells per microliter) were observed in 3.6% of MAVENCLAD-treated patients, compared to 2.8% of placebo patients. Decreases in hemoglobin levels, in general mild to moderate (hemoglobin 8.0 g per dL to < LLN), were observed in 26% of MAVENCLAD-treated patients, compared to 19% of placebo patients. Decreases in platelet counts were generally mild (cell count 75,000 cells per microliter to < LLN) and were observed in 11% of MAVENCLAD-treated patients, compared to 4% of placebo patients.

In clinical studies at dosages similar to or higher than the approved MAVENCLAD dosage, serious cases of thrombocytopenia, neutropenia, and pancytopenia (some with documented bone marrow hypoplasia) requiring transfusion and granulocyte-colony stimulating factor treatment have been reported *[see Warnings and Precautions (5.6)* for information regarding graft-versus-host disease with blood transfusion*]*.

Obtain complete blood count (CBC) with differential prior to, during, and after treatment with MAVENCLAD *[see Dosage and Administration (2.1, 2.5)]*.

5.6 Graft-Versus-Host Disease With Blood Transfusion

Transfusion-associated graft-versus-host disease has been observed rarely after transfusion of nonirradiated blood in patients treated with cladribine for non-MS treatment indications.

In patients who require blood transfusion, irradiation of cellular blood components is recommended prior to administration to decrease the risk of transfusion-related graft-versus-host disease. Consultation with a hematologist is advised.

5.7 Liver Injury

In clinical studies, 0.3% of MAVENCLAD-treated patients had liver injury (serious or causing treatment discontinuation) considered related to treatment, compared to 0 placebo patients. Onset has ranged from a few weeks to several months after initiation of treatment with MAVENCLAD. Signs and symptoms of liver injury, including elevation of serum aminotransferases to greater than 20-fold the upper limit of normal, have been observed. These abnormalities resolved upon treatment discontinuation.

Obtain serum aminotransferase, alkaline phosphatase, and total bilirubin levels prior to the first and second treatment course *[see Dosage and Administration (2.1)]*. If a patient develops clinical signs, including unexplained liver enzyme elevations or symptoms suggestive of hepatic dysfunction (e.g., unexplained nausea, vomiting, abdominal pain, fatigue, anorexia, or jaundice and/or dark urine), promptly measure serum transaminases and total bilirubin and interrupt or discontinue treatment with MAVENCLAD, as appropriate.

5.8 Hypersensitivity

In clinical studies, 11% of MAVENCLAD-treated patients had hypersensitivity reactions, compared to 7% of placebo patients. Hypersensitivity reactions that were serious and/or led to discontinuation of MAVENCLAD (e.g., dermatitis, pruritis) occurred in 0.5% of MAVENCLAD-treated patients, compared to 0.1% of placebo patients. One patient had a serious hypersensitivity reaction with rash, mucous membrane ulceration, throat swelling, vertigo, diplopia, and headache after the first dose of MAVENCLAD.

If a hypersensitivity reaction is suspected, discontinue MAVENCLAD therapy. Do not use MAVENCLAD in patients with a history of hypersensitivity to cladribine *[see Contraindications (4)]*.

5.9 Cardiac Failure

In clinical studies, one MAVENCLAD-treated patient experienced life-threatening acute cardiac failure with myocarditis, which improved after approximately one week. Cases of cardiac failure have also been reported with parenteral cladribine used for treatment indications other than multiple sclerosis.

Instruct patients to seek medical advice if they experience symptoms of cardiac failure (e.g., shortness of breath, rapid or irregular heartbeat, swelling).

6 ADVERSE REACTIONS

The following serious adverse reactions and potential risks are discussed, or discussed in greater detail, in other sections of the labeling:

- Malignancies *[see Warnings and Precautions (5.1)]*
- Risk of Teratogenicity *[see Warnings and Precautions (5.2)]*
- Lymphopenia *[see Warnings and Precautions (5.3)]*
- Infections *[see Warnings and Precautions (5.4)]*
- Hematologic Toxicity *[see Warnings and Precautions (5.5)]*
- Graft-Versus-Host Disease With Blood Transfusion *[see Warnings and Precautions (5.6)]*
- Liver Injury *[see Warnings and Precautions (5.7)]*
- Hypersensitivity *[see Warnings and Precautions (5.8)]*
- Cardiac Failure *[see Warnings and Precautions (5.9)]*

6.1 Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical studies of another drug and may not reflect the rates observed in practice.

In the clinical trial program of cladribine in MS, 1,976 patients received cladribine for a total of 9,509 patient years. The mean time on study including follow-up was approximately 4.8 years, and approximately 24% of cladribine-treated patients had approximately 8 years of time on study including follow-up. Of these, 923 patients aged 18 to 66 years received MAVENCLAD as monotherapy at a cumulative dose of 3.5 mg per kg.

Table 2 shows adverse reactions in Study 1 *[see Clinical Studies (14)]* with an incidence of at least 5% for MAVENCLAD and higher than placebo. The most common (> 20%) adverse reactions reported in Study 1 are upper respiratory tract infection, headache, and lymphopenia.

Table 2 **Adverse Reactions in Study 1 with an Incidence of at Least 5% for MAVENCLAD and Higher than Placebo**

	MAVENCLAD (N=440) %	Placebo (N=435) %
Upper respiratory tract infection	38	32
Headache	25	19
Lymphopenia	24	2
Nausea	10	9
Back pain	8	6
Arthralgia and arthritis	7	5
Insomnia	6	4
Bronchitis	5	3
Hypertension	5	3
Fever	5	3
Depression	5	3

Hypersensitivity
In clinical studies, 11% of MAVENCLAD patients had hypersensitivity adverse reactions, compared to 7% of placebo patients *[see Warnings and Precautions (5.8)]*.

Alopecia
Alopecia occurred in 3% of MAVENCLAD-treated patients compared to 1% of placebo patients.

Myelodysplastic Syndrome
Cases of myelodysplastic syndrome have been reported in patients that had received parenteral cladribine at a higher dosage than that approved for MAVENCLAD. These cases occurred several years after treatment.

Herpes Meningoencephalitis
Fatal herpes meningoencephalitis occurred in one MAVENCLAD-treated patient, at a higher dosage and longer duration of therapy than the approved MAVENCLAD dosage and in combination with interferon beta-1a treatment.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)
SJS and TEN are identified risks of parenteral cladribine for the treatment of oncologic indications.

Seizures
In clinical studies, serious events of seizure occurred in 0.3% of MAVENCLAD-treated patients compared to 0 placebo patients. Serious events included generalized tonic-clonic seizures and status epilepticus. It is unknown whether these events were related to the effects of multiple sclerosis alone, to MAVENCLAD, or to a combination of both.

7 DRUG INTERACTIONS

Table 3 Drug Interactions with MAVENCLAD

7.1 Immunomodulatory, Immunosuppressive, or Myelosuppressive Drugs	
Clinical Impact	Concomitant use of MAVENCLAD with immunomodulatory, immunosuppressive, or myelosuppressive drugs may increase the risk of adverse reactions because of the additive effects on the immune system *[see Warnings and Precautions (5.4)]*.
Prevention or Management	Concomitant use with myelosuppressive or other immunosuppressive drugs is not recommended. Acute short-term therapy with corticosteroids can be administered. In patients who have previously been treated with immunomodulatory or immunosuppressive drugs, consider potential additive effect, the mode of action, and duration of effect of the other drugs prior to initiation of MAVENCLAD.
7.2 Interferon-Beta	
Clinical Impact	Concomitant use of MAVENCLAD with interferon-beta did not change the exposure of cladribine to a clinically significant effect; however, lymphopenia risk may be increased *[see Warnings and Precautions (5.3)]*.
Prevention or Management	Concomitant use is not recommended.
7.3 Hematotoxic Drugs	
Clinical Impact	Concomitant use of MAVENCLAD with hematotoxic drugs may increase the risk of adverse reactions because of the additive hematological effects *[see Warnings and Precautions (5.5)]*.
Prevention or Management	Monitor hematological parameters.
7.4 Antiviral and Antiretroviral Drugs	
Clinical Impact	Compounds that require intracellular phosphorylation to become active (e.g., lamivudine, zalcitabine, ribavirin, stavudine, and zidovudine) could interfere with the intracellular phosphorylation and activity of cladribine.
Prevention or Management	Avoid concomitant use.

7.5 Potent ENT, CNT and BCRP Transporter Inhibitors	
Clinical Impact	Cladribine is a substrate of breast cancer resistance protein (BCRP), equilibrative nucleoside (ENT1), and concentrative nucleoside (CNT3) transport proteins. The bioavailability, intracellular distribution, and renal elimination of cladribine may be altered by potent ENT1, CNT3, and BCRP transporter inhibitors.
Prevention or Management	Avoid co-administration of potent ENT1, CNT3, or BCRP transporter inhibitors (e.g., ritonavir, eltrombopag, curcumin, cyclosporine, dilazep, nifedipine, nimodipine, cilostazol, sulindac, dipyridamole, or reserpine) during the 4 to 5 day MAVENCLAD treatment cycles. If this is not possible, consider selection of alternative concomitant drugs with no or minimal ENT1, CNT3, or BCRP transporter inhibiting properties. If this is not possible, dose reduction to the minimum mandatory dose of drugs containing these compounds, separation in the timing of administration, and careful patient monitoring is recommended.
7.6 Potent BCRP and P-gp Transporter Inducers	
Clinical Impact	Possible decrease in cladribine exposure if potent BCRP or P-gp transporter inducers are co-administered.
Prevention or Management	Consider a possible decrease in cladribine efficacy if potent BCRP (e.g., corticosteroids) or P-gp (e.g., rifampicin, St. John's Wort) transporter inducers are co-administered.
7.7 Hormonal Contraceptives	
Clinical Impact	It is currently unknown whether MAVENCLAD may reduce the effectiveness of systemically acting hormonal contraceptives.
Prevention or Management	Women using systemically acting hormonal contraceptives should add a barrier method during MAVENCLAD dosing and for at least 4 weeks after the last dose in each treatment course.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

MAVENCLAD is contraindicated in pregnant women and in females and males of reproductive potential who do not plan to use effective contraception. There are no adequate data on the developmental risk associated with use of MAVENCLAD in pregnant women. Cladribine was embryolethal when administered to pregnant mice and produced malformations in mice and rabbits *[see Data]*. The observed developmental effects are consistent with the effects of cladribine on DNA *[see Contraindications (4) and Warnings and Precautions (5.2)]*.

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. The background risk of major birth defects and miscarriage for the indicated population is unknown.

Data

Animal Data
When cladribine was administered intravenously (0, 0.5, 1.5, or 3 mg/kg/day) to pregnant mice during the period of organogenesis, fetal growth retardation and malformations (including exencephaly and cleft palate) and embryofetal death were observed at the highest dose tested. An increase in skeletal variations was observed at all but the lowest dose tested. There was no evidence of maternal toxicity.

When cladribine was administered intravenously (0, 0.3, 1, and 3 mg/kg/day) to pregnant rabbits during the period of organogenesis, fetal growth retardation and a high incidence of craniofacial and limb malformations were observed at the highest dose tested, in the absence of maternal toxicity.

When cladribine was administered intravenously (0, 0.5, 1.5, or 3.0 mg/kg/day) to mice throughout pregnancy and lactation, skeletal anomalies and embryolethality were observed at all but the lowest dose tested.

8.2 Lactation

Risk Summary

MAVENCLAD is contraindicated in breastfeeding women because of the potential for serious adverse reactions in breastfed infants *[see Contraindications (4) and Warnings and Precautions (5)]*. Advise women not to breastfeed during dosing with MAVENCLAD and for 10 days after the last dose.

There are no data on the presence of cladribine in human milk, the effects on the breastfed infant, or the effects of the drug on milk production.

8.3 Females and Males of Reproductive Potential

Pregnancy Testing

In females of reproductive potential, pregnancy should be excluded before the initiation of each treatment course of MAVENCLAD *[see Use in Specific Populations (8.1)]*.

Contraception

Females
Females of reproductive potential should prevent pregnancy by use of effective contraception during MAVENCLAD dosing and for at least 6 months after the last dose in each treatment course. It is unknown if MAVENCLAD may reduce the effectiveness of the systemically acting hormonal contraceptives. Women using systemically acting hormonal contraceptives should add a barrier method during MAVENCLAD dosing and for at least 4 weeks after the last dose in each treatment course. Women who become pregnant during MAVENCLAD therapy should discontinue treatment *[see Warnings and Precautions (5.2) and Drug Interactions (7.7)]*.

Males
As cladribine interferes with DNA synthesis, adverse effects on human gametogenesis could be expected. Therefore, male patients of reproductive potential should take precautions to prevent pregnancy of their partner during MAVENCLAD dosing and for at least 6 months after the last dose in each treatment course *[see Warnings and Precautions (5.2) and Nonclinical Toxicology (13.1)]*.

8.4 Pediatric Use

The safety and effectiveness in pediatric patients (below 18 years of age) have not been established. Use of MAVENCLAD is not recommended in pediatric patients because of the risk of malignancies *[see Warnings and Precautions (5.1)]*.

8.5 Geriatric Use

Clinical studies with MAVENCLAD did not include sufficient numbers of patients aged 65 and over to determine whether they respond differently from younger patients. Other reported clinical experience has not identified differences in responses between the elderly and younger patients. Caution is recommended when MAVENCLAD is used in elderly patients, taking into account the potential greater frequency of decreased hepatic, renal, or cardiac function, concomitant diseases, and other drug therapy.

8.6 Patients with Renal Impairment

The concentration of cladribine is predicted to increase in patients with renal impairment *[see Clinical Pharmacology (12.3)]*. No dosage adjustment is recommended in patients with mild renal impairment (creatinine clearance 60 to 89 mL per minute). MAVENCLAD is not recommended in patients with moderate to severe renal impairment (creatinine clearance below 60 mL per minute) *[see Clinical Pharmacology (12.3)]*.

8.7 Patients with Hepatic Impairment

The effect of hepatic impairment on the pharmacokinetics of cladribine is unknown *[see Clinical Pharmacology (12.3)]*. No dosage adjustment is recommended in patients with mild hepatic impairment. MAVENCLAD is not recommended in patients with moderate to severe hepatic impairment (Child-Pugh score greater than 6) *[see Clinical Pharmacology (12.3)]*.

10 OVERDOSAGE

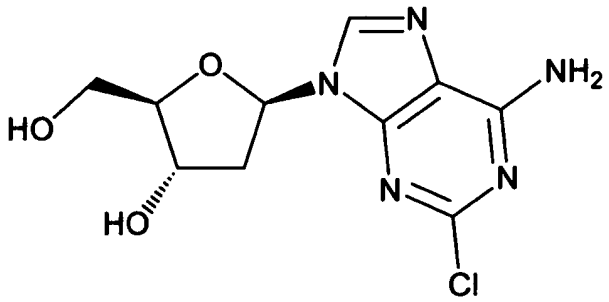
There is no experience with overdose of MAVENCLAD. Lymphopenia is known to be dose-dependent. Particularly close monitoring of hematological parameters is recommended in patients who have been exposed to an overdose of MAVENCLAD *[see Warnings and Precautions (5.3, 5.5)]*.

There is no known specific antidote to an overdose of MAVENCLAD. Treatment consists of careful observation and initiation of appropriate supportive measures. Discontinuation of MAVENCLAD may need to be considered. Because of the rapid and extensive intracellular and tissue distribution, hemodialysis is unlikely to eliminate cladribine to a significant extent.

11 DESCRIPTION

MAVENCLAD contains the nucleoside metabolic inhibitor cladribine, which is a white or almost white, non-hydroscopic, crystalline powder with the molecular formula $C_{10}H_{12}ClN_5O_3$ and molecular weight 285.69. It differs in structure from the naturally occurring nucleoside, deoxyadenosine, by the substitution of chlorine for hydrogen in the 2-position of the purine ring.

The chemical name of cladribine is 2-chloro-2′-deoxy-adenosine. The structural formula is shown below:



Cladribine is stable at slightly basic and at neutral pH. The main degradation pathway is hydrolysis and at acidic pH significant decomposition occurs with time. The ionization behavior of the molecule over the pH range 0 to 12 is characterized by a single pKa of approximately 1.21.

MAVENCLAD is provided as 10 mg tablets for oral use. Each MAVENCLAD 10 mg tablet contains cladribine as an active ingredient and hydroxypropyl betadex, magnesium stearate, and sorbitol as inactive ingredients.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The mechanism by which cladribine exerts its therapeutic effects in patients with multiple sclerosis has not been fully elucidated but is thought to involve cytotoxic effects on B and T lymphocytes through impairment of DNA synthesis, resulting in depletion of lymphocytes.

12.2 Pharmacodynamics

MAVENCLAD causes a dose-dependent reduction in lymphocyte count. The lowest absolute lymphocyte counts occurred approximately 2 to 3 months after the start of each treatment cycle and were lower with each additional treatment cycle. At the end of Year 2, 2% of patients continued to have absolute lymphocyte counts less than 500 cells per microliter. The median time to recovery from lymphocyte counts less than 500 cells per microliter to at least 800 cells per microliter was approximately 28 weeks *[see Warnings and Precautions (5.3)]*.

12.3 Pharmacokinetics

Cladribine is a prodrug that becomes active upon phosphorylation to its 2-chlorodeoxyadenosine triphosphate (Cd-ATP) metabolite.

The pharmacokinetic parameters presented below were assessed following oral administration of cladribine 10 mg, unless otherwise specified. The cladribine mean maximum concentration (C_{max}) was in the range of 22 to 29 ng/ mL and corresponding mean AUC was in the range of 80 to 101 ng•h/mL.

The C_{max} and AUC of cladribine increased proportionally across a dose range from 3 to 20 mg.

No accumulation of cladribine concentration in plasma was observed after repeated dosing.

Absorption

The bioavailability of cladribine was approximately 40%. Following fasted administration of cladribine, the median time to maximum concentration (T_{max}) was 0.5 h (range 0.5 to 1.5 hours).

Effect of Food
Following administration of cladribine with a high fat meal, the geometric mean C_{max} decreased by 29% and AUC was unchanged. The T_{max} was prolonged to 1.5 hours (range 1 to 3 hours). This difference is not expected to be clinically significant.

Distribution

Cladribine mean apparent volume of distribution ranges from 480 to 490 liters. The plasma protein binding of cladribine is 20% and is independent of concentration, in vitro.

Intracellular concentrations of cladribine and/or its metabolites in human lymphocytes were approximately 30 to 40 times extracellular, in vitro.

Cladribine has the potential to penetrate the blood brain barrier. A cerebrospinal fluid/plasma concentration ratio of approximately 0.25 was observed in cancer patients.

Elimination

Cladribine estimated terminal half-life is approximately 1 day. The intracellular half-life of the cladribine phosphorylated metabolites cladribine monophosphate (Cd-AMP) is 15 hours and Cd-ATP is 10 hours. Cladribine estimated median apparent renal clearance is 22.2 liter per hour and non-renal clearance is 23.4 liter per hour.

Metabolism
Cladribine is a prodrug that is phosphorylated to Cd-AMP by deoxycytidine kinase (and also by deoxyguanosine kinase in the mitochondria) in lymphocytes. Cd-AMP is further phosphorylated to cladribine diphosphate (Cd-ADP) and the active moiety Cd-ATP. The dephosphorylation and deactivation of Cd-AMP is catalyzed by cytoplasmic 5'-nucleotidase (5'-NTase).

The metabolism of cladribine in whole blood has not been fully characterized. However, extensive whole blood and negligible hepatic enzyme metabolism was observed, in vitro.

Excretion
After administration of 10 mg oral cladribine in MS patients, 28.5 [20] (mean [SD]) percent of the dose was excreted unchanged via the renal route. Renal clearance exceeded the glomerular filtration rate, indicating active renal secretion of cladribine.

Specific Populations

No studies have been conducted to evaluate the pharmacokinetics of cladribine in elderly or in patients with renal or hepatic impairment.

There were no clinically significant differences in the pharmacokinetics of cladribine based on age (range 18 to 65 years) or gender. The effect of hepatic impairment on the pharmacokinetics of cladribine is unknown.

Patients with Renal Impairment
Renal clearance of cladribine was shown to be dependent on creatinine clearance (CL_{CR}). No dedicated studies have been conducted in patients with renal impairment, however patients with mild renal impairment (CL_{CR} of 60 mL to below 90 mL per minute) were included in Study 1. A pooled pharmacokinetic analysis estimated a decrease of 18% in total clearance in a typical subject with a CL_{CR} of 65 mL per minute leading to an increase in cladribine exposure of 25%. Clinical experience in patients with moderate to severe renal impairment (i.e., CL_{CR} below 60 mL per minute) is limited *[see Use in Specific Populations (8.6)]*.

Drug Interaction Studies

Clinical Studies
No clinically significant differences in cladribine pharmacokinetics were observed when used concomitantly with pantoprazole or interferon beta-1a.

In Vitro Studies
It has been reported that lamivudine can inhibit the phosphorylation of cladribine intracellularly. Potential competition for intracellular phosphorylation exists between cladribine and compounds that require intracellular phosphorylation to become active (e.g., lamivudine, zalcitabine, ribavirin, stavudine, and zidovudine).

Cytochrome P450 (CYP) Enzymes: Cladribine is not a substrate of cytochrome P450 enzymes and does not show significant potential to act as inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. Cladribine has no clinically meaningful inductive effect on CYP1A2, CYP2B6 and CYP3A4 enzymes.

Transporter Systems: Cladribine is a substrate of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), equilibrative nucleoside transporter 1 (ENT1) and concentrative nucleoside transporter 3 (CNT3). Inhibition of BCRP in the gastrointestinal tract may increase the oral bioavailability and systemic exposure of cladribine. Intracellular distribution and renal elimination of cladribine may be altered by potent ENT1, CNT3 transporter inhibitors.

12.6 Hydroxypropyl Betadex-Related Complex Formation

MAVENCLAD contains hydroxypropyl betadex that may be available for complex formation with the active ingredients of other drugs. Complex formation between free hydroxypropyl betadex, released from the cladribine tablet formulation, and concomitant ibuprofen, furosemide, and gabapentin was observed. Concomitant use with MAVENCLAD may increase the bioavailability of other drugs (especially agents with low solubility), which may increase the risk or severity of adverse reactions *[see Dosage and Administration (2.4)]*.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

In mice administered cladribine (0, 0.1, 1, or 10 mg/kg) by subcutaneous injection intermittently (7 daily doses followed by 21 days of non-dosing per cycle) for 22 months, an increase in Harderian gland tumors (adenoma) was observed at the highest dose tested.

Mutagenesis

Cladribine was negative for mutagenicity in in vitro (reverse mutation in bacteria, CHO/HGPRT mammalian cell) assays.

Cladribine was positive for clastogenicity in an in vitro mammalian cell assay, in the absence and presence of metabolic activation, and in an in vivo mouse micronucleus assay.

Impairment of Fertility

When cladribine (0, 1, 5, 10, or 30 mg/kg/day) was administered by subcutaneous injection to male mice prior to and during mating to untreated females, no effects on fertility were observed. However, an increase in non-motile sperm was observed at the highest dose tested. In female mice, administration of cladribine (0, 1, 2, 4, or 8 mg/kg/day) by subcutaneous injection prior to and during mating to untreated males and continuing to gestation day 6 caused an increase in embryolethality at the highest dose tested.

In monkeys administered cladribine (0, 0.15, 0.3, or 1.0 mg/kg) by subcutaneous injection intermittently (7 consecutive daily doses followed by 21 days of non-dosing per cycle) for one year, testicular degeneration was observed at the highest dose tested.

14 CLINICAL STUDIES

The efficacy of MAVENCLAD was demonstrated in a 96-week randomized, double-blind, placebo-controlled clinical study in patients with relapsing forms of MS (Study 1; NCT00213135).

Patients were required to have at least 1 relapse in the previous 12 months. The median age was 39 years (range 18 to 65) and the female-to-male ratio was approximately 2:1. The mean duration of MS prior to study enrollment was 8.7 years, and the median baseline neurological disability based on Kurtzke Expanded Disability Status Scale (EDSS) score across all treatment groups was 3.0. Over two thirds of the study patients were treatment-naive for drugs used to treat relapsing forms of MS.

1,326 patients were randomized to receive either placebo (n = 437), or a cumulative oral dosage of MAVENCLAD 3.5 mg per kg (n = 433) or 5.25 mg per kg body weight (n = 456) over the 96-week study period in 2 treatment courses. Patients randomized to the 3.5 mg per kg cumulative dose received a first treatment course at Weeks 1 and 5 of the first year and a second treatment course at Weeks 1 and 5 of the second year *[see Dosage and Administration (2.2)]*. Patients randomized to the 5.25 mg per kg cumulative dose received additional treatment at Weeks 9 and 13 of the first year. Higher cumulative doses did not add any clinically meaningful benefit, but were associated with a higher incidence in grade 3 lymphopenia or higher (44.9% in the 5.25 mg per kg group vs. 25.6% in the 3.5 mg per kg group). Ninety-two percent of patients treated with MAVENCLAD 3.5 mg per kg and 87% of patients receiving placebo completed the full 96 weeks of the study.

The primary outcome of Study 1 was the annualized relapse rate (ARR). Additional outcome measures included the proportion of patients with confirmed disability progression, the time to first qualifying relapse, the mean number of MRI T1 Gadolinium-enhancing (Gd+) lesions, and new or enlarging MRI T2 hyperintense lesions. Disability progression was measured in terms of a 3-month sustained change in EDSS score of at least one point, if baseline EDSS score was between 0.5 and 4.5 inclusively, or at least 1.5 points if the baseline EDSS score was 0, or at least 0.5 point if the baseline EDSS score was at least 5, over a period of at least 3 months.

MAVENCLAD 3.5 mg per kg significantly lowered the annualized relapse rate. The results from Study 1 are presented in Table 4.

Table 4 Clinical Outcomes in Study 1 (96 Weeks) - Primary and Secondary Endpoints

Endpoints	MAVENCLAD Cumulative Dose 3.5 mg per kg (n = 433)	Placebo (n = 437)
Clinical Endpoints		
Annualized relapse rate (ARR)	0.14*	0.33
Relative reduction in ARR	58%	
Proportion of patients without relapse	81%**	63%
Time to 3-month confirmed EDSS progression, HR	0.67**	
Proportion of patients with 3-month EDSS progression	13%	19%
MRI Endpoints		
Median Number of Active T1 Gd+ Lesions	0*	0.33
Median Number of Active T2 Lesions	0*	0.67

* $p < 0.001$ compared to placebo
** nominal $p < 0.05$ compared to placebo
HR: Hazard Ratio

15 REFERENCES

1 "OSHA Hazardous Drugs". OSHA. http://www.osha.gov/SLTC/hazardousdrugs/index.html.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

MAVENCLAD tablets, 10 mg, are uncoated, white, round, biconvex, and engraved with a "C" on one side and "10" on the other side. Each tablet is packaged in a child-resistant day pack containing one or two tablets in a blister card.

Dispense one box for each treatment cycle with a Medication Guide *[see Dosage and Administration (2.2)]*.

Presentations

NDC 44087-400-11	Box of 1 tablet: One day pack containing one tablet.
NDC 44087-400-12	Box of 2 tablets: One day pack containing two tablets.
NDC 44087-400-04	Box of 4 tablets: Four day packs each containing one tablet.
NDC 44087-400-05	Box of 5 tablets: Five day packs each containing one tablet.
NDC 44087-400-06	Box of 6 tablets: One day pack containing two tablets. Four day packs each containing one tablet.
NDC 44087-400-07	Box of 7 tablets: Two day packs each containing two tablets. Three day packs each containing one tablet.
NDC 44087-400-08	Box of 8 tablets: Three day packs each containing two tablets. Two day packs each containing one tablet.
NDC 44087-400-09	Box of 9 tablets: Four day packs each containing two tablets. One day pack containing one tablet.
NDC 44087-400-10	Box of 10 tablets: Five day packs each containing two tablets.

16.2 Storage and Handling

Store at controlled room temperature, 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F) *[see USP Controlled Room Temperature]*. Store in original package in order to protect from moisture.

MAVENCLAD is a cytotoxic drug. Follow applicable special handling and disposal procedures *[see References (15)]*.[1]

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Medication Guide).

Malignancies

Inform patients that MAVENCLAD may increase their risk of malignancies. Instruct patients to follow standard cancer screening guidelines *[see Dosage and Administration (2) and Warnings and Precautions (5.1)]*.

Risk of Teratogenicity

Inform patients that MAVENCLAD may cause fetal harm. Discuss with women of childbearing age whether they are pregnant, might be pregnant, or are trying to become pregnant. Before initiating each treatment course, inform patients about the potential risk to the fetus, if female patients or partners of male patients get pregnant during MAVENCLAD dosing or within 6 months after the last dose in each treatment course *[see Warnings and Precautions (5.2) and Use in Specific Populations (8.1, 8.3)]*.

Instruct female patients of childbearing potential to use effective contraception during MAVENCLAD dosing and for at least 6 months after the last dose in each treatment course to avoid pregnancy. Advise women using systemically acting hormonal contraceptives to add a barrier method during MAVENCLAD dosing and for at least 4 weeks after the last dose in each treatment course because MAVENCLAD may reduce the effectiveness of the hormonal contraceptive *[see Drug Interactions (7.7)]*.

Instruct male patients to take precautions to prevent pregnancy of their partner during MAVENCLAD dosing and for at least 6 months after the last dose in each treatment course.

Advise patients that female patients or partners of male patients who get pregnant immediately inform their healthcare provider.

Lactation

Inform women that they cannot breastfeed on a MAVENCLAD treatment day and for 10 days after the last dose *[see Use in Specific Populations (8.2)]*.

Lymphopenia and Other Hematologic Toxicity

Inform patients that MAVENCLAD may decrease lymphocyte counts and may also decrease counts of other blood cells. A blood test should be obtained before starting a treatment course, 2 and 6 months after start of treatment in each treatment course, periodically thereafter, and when clinically needed. Advise patients to keep all appointments for lymphocyte monitoring during and after MAVENCLAD treatment *[see Dosage and Administration (2.5) and Warnings and Precautions (5.3, 5.5)]*.

Infections

Inform patients that use of MAVENCLAD may increase the risk of infections. Instruct patients to notify their healthcare provider promptly if fever or other signs of infection such as aching, painful muscles, headache, generally feeling unwell or loss of appetite occur while on therapy or after a course of treatment *[see Warnings and Precautions (5.4)]*.

Advise patients that PML has happened with parenteral cladribine used in oncologic indications. Inform the patient that PML is characterized by a progression of deficits and usually leads to death or severe disability over weeks or months. Instruct the patient of the importance of contacting their doctor if they develop any symptoms suggestive of PML. Inform the patient that typical symptoms associated with PML are diverse, progress over days to weeks, and include progressive weakness on one side of the body or clumsiness of limbs, disturbance of vision, and changes in thinking, memory, and orientation leading to confusion and personality changes *[see Warnings and Precautions (5.4)]*.

Liver Injury

Inform patients that MAVENCLAD may cause liver injury. Instruct patients treated with MAVENCLAD to report promptly any symptoms that may indicate liver injury, including fatigue, anorexia, right upper abdominal discomfort, dark urine, or jaundice. A blood test should be obtained prior to each treatment course with MAVENCLAD and as clinically indicated thereafter *[see Warnings and Precautions (5.7)]*.

Hypersensitivity

Advise patients to seek immediate medical attention if they experience any symptoms of serious or severe hypersensitivity reactions, including skin reactions *[see Warnings and Precautions (5.8)]*.

Cardiac Failure

Advise patients that MAVENCLAD may cause cardiac failure. Instruct patients to seek medical advice if they experience symptoms of cardiac failure (e.g., shortness of breath, rapid or irregular heartbeat, swelling) *[see Warnings and Precautions (5.9)]*.

Treatment Handling and Administration

Instruct patients that MAVENCLAD is a cytotoxic drug and to use care when handling MAVENCLAD tablets, limit direct skin contact with the tablets, and wash exposed areas thoroughly. Advise patients to keep the tablets in the original package until just prior to each scheduled dose and consult their pharmacist on the proper disposal of unused tablets *[see Dosage and Administration (2.4) and How Supplied/Storage and Handling (16.2)]*.

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Rockland, MA 02370

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MEDICATION GUIDE

MAVENCLAD® (MAY-ven-klad)
(cladribine)
tablets, for oral use

What is the most important information I should know about MAVENCLAD?

MAVENCLAD can cause serious side effects, including:

- **Risk of cancer (malignancies).** Treatment with MAVENCLAD may increase your risk of developing cancer. Talk to your healthcare provider about your risk of developing cancer if you receive MAVENCLAD. You should follow your healthcare provider instructions about screening for cancer.
- **MAVENCLAD may cause birth defects if used during pregnancy. Females must not be pregnant when they start treatment with MAVENCLAD or become pregnant during MAVENCLAD dosing and within 6 months after the last dose of each yearly treatment course. Stop your treatment with MAVENCLAD and call your healthcare provider right away if you become pregnant during treatment with MAVENCLAD.**
  - For females who are able to become pregnant:
    - Your healthcare provider should order a pregnancy test for you before you begin your first and second yearly treatment course of MAVENCLAD to make sure that you are not pregnant. Your healthcare provider will decide when to do the test.
    - Use effective birth control (contraception) on the days on which you take MAVENCLAD and for at least 6 months after the last dose of each yearly treatment course.
      - Talk to your healthcare provider if you use oral contraceptives (the "pill").
      - You should use a second method of birth control on the days on which you take MAVENCLAD and for at least 4 weeks after your last dose of each yearly treatment course.
  - For males with female partners who are able to become pregnant:
    - Use effective birth control (contraception) during the days on which you take MAVENCLAD and for at least 6 months after the last dose of each yearly treatment course.

What is MAVENCLAD?

MAVENCLAD is a prescription medicine used to treat relapsing forms of multiple sclerosis (MS), to include relapsing-remitting disease and active secondary progressive disease, in adults. Because of its safety profile, MAVENCLAD is generally used in people who have tried another MS medicine that they could not tolerate or that has not worked well enough.

MAVENCLAD is not recommended for use in people with clinically isolated syndrome (CIS).

It is not known if MAVENCLAD is safe and effective in children under 18 years of age.

Do not take MAVENCLAD if you:

- have cancer (malignancy).
- are pregnant, plan to become pregnant, or are a woman of childbearing age or a man able to father a child and you are not using birth control. See "**What is the most important information I should know about MAVENCLAD?**"
- are human immunodeficiency virus (HIV) positive.
- have active infections, including tuberculosis (TB), hepatitis B or C.
- are allergic to cladribine.
- are breastfeeding. See "**Before you take MAVENCLAD, tell your healthcare provider about all of your medical conditions, including if you:**"

Before you take MAVENCLAD, tell your healthcare provider about all of your medical conditions, including if you:

- think you have an infection.
- have heart failure.
- have liver or kidney problems.
- have taken, take, or plan to take medicines that affect your immune system or your blood cells, or other treatments for MS. Certain medicines can increase your risk of getting an infection.
- have had a recent vaccination or are scheduled to receive any vaccinations. You should not receive live or live-attenuated vaccines within the 4 to 6 weeks preceding your treatment with MAVENCLAD. You should not receive these types of vaccines during your treatment with MAVENCLAD and until your healthcare provider tells you that your immune system is no longer weakened.
- have or have had cancer.
- are breastfeeding or plan to breastfeed. It is not known if MAVENCLAD passes into your breast milk. Do not breastfeed on the days, on which you take MAVENCLAD, and for 10 days after the last dose. See "**Do not take**

MAVENCLAD if you:"

Tell your healthcare provider about all the medicines you take, including prescription and over-the-counter medicines, vitamins, and herbal supplements.

How should I take MAVENCLAD?

- MAVENCLAD is given as two yearly treatment courses.
- Each yearly treatment course consists of 2 treatment weeks (also called cycles) that will be about a month apart. Your healthcare provider will tell you when you have to start your treatment weeks and how many tablets per week you need, depending on your weight. Each treatment week is 4 or 5 days.
- Your pharmacist will dispense a carton of MAVENCLAD for each treatment week. The prescribed number of tablets per day are provided in child resistant day packs.
- Take MAVENCLAD exactly as your healthcare provider tells you. Do not change your dose or stop taking MAVENCLAD unless your healthcare provider tells you to.
- Take MAVENCLAD with water and swallow whole without chewing. MAVENCLAD can be taken with or without food.
- Swallow MAVENCLAD right away after opening the blister pack.
- Your hands must be dry when handling MAVENCLAD and washed well with water afterwards.
- Limit contact with your skin. Avoid touching your nose, eyes and other parts of the body. If you get MAVENCLAD on your skin or on any surface, wash it right away with water.
- Take MAVENCLAD at least 3 hours apart from other medicines taken by mouth during the 4- to 5-day MAVENCLAD treatment week.
- If you miss a dose, take it as soon as you remember on the same day. If the whole day passes before you remember, take your missed dose the next day. **Do not take 2 doses at the same time**. Instead, you will extend the number of days in that treatment week.

Your healthcare provider will continue to monitor your health during the 2 yearly treatment courses, and for at least another 2 years during which you do not need to take MAVENCLAD. It is not known if MAVENCLAD is safe and effective in people who restart MAVENCLAD treatment more than 2 years after completing 2 yearly treatment courses.

What are the possible side effects of MAVENCLAD?

MAVENCLAD can cause serious side effects, including:

- See **"What is the most important information I should know about MAVENCLAD?"**
- **low blood cell counts**. Low blood cell counts have happened and can increase your risk of infections during your treatment with MAVENCLAD. Your healthcare provider will do blood tests before you start treatment with MAVENCLAD, during your treatment with MAVENCLAD, and afterward, as needed.
- **serious infections such as:**
  - **TB, hepatitis B or C, and shingles (herpes zoster).** Fatal cases of TB and hepatitis have happened with cladribine during clinical studies. Tell your healthcare provider right away if you get any symptoms of the following infection related problems or if any of the symptoms get worse, including:
    - fever
    - aching painful muscles
    - headache
    - feeling of being generally unwell
    - loss of appetite
    - burning, tingling, numbness or itchiness of the skin in the affected area
    - skin blotches, blistered rash and severe pain
  - **progressive multifocal leukoencephalopathy (PML).** PML is a rare brain infection that usually leads to death or severe disability. Although PML has not been seen in MS patients taking MAVENCLAD, it may happen in people with weakened immune systems. Symptoms of PML get worse over days to weeks. Call your healthcare provider right away if you have any new or worsening neurologic signs or symptoms of PML, that have lasted several days, including:
    - weakness on 1 side of your body
    - loss or coordination in your arms and legs
    - decreased strength
    - problems with balance
    - changes in your vision
    - changes in your thinking or memory
    - confusion
    - changes in your personality
- **liver problems.** MAVENCLAD may cause liver problems. Your healthcare provider should do blood tests to check your liver before you start taking MAVENCLAD. Call your healthcare provider right away if you have any of the following symptoms of liver problems:
  - nausea
  - vomiting
  - stomach pain
  - tiredness
  - loss of appetite
  - your skin or the whites or your eyes turn yellow
  - dark urine

- **allergic reactions (hypersensitivities)**. MAVENCLAD can cause serious allergic reactions. Stop your treatment with MAVENCLAD and go to the closest emergency room for medical help right away if you have any signs or symptoms of allergic reactions. Symptoms of an allergic reaction may include: skin rash, swelling or itching of the face, lips, tongue or throat, or trouble breathing.
- **heart failure**. MAVENCLAD may cause heart failure, which means your heart may not pump as well as it should. Call your healthcare provider or go to the closest emergency room for medical help right away if you have any signs or symptoms such as shortness of breath, a fast or irregular heart beat, or unusual swelling in your body.

Your healthcare provider may delay or completely stop treatment with MAVENCLAD if you have severe side effects.

The most common side effects of MAVENCLAD include:

- upper respiratory infection
- headache
- low white blood cell counts

These are not all the possible side effects of MAVENCLAD. Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

How should I store MAVENCLAD?

- MAVENCLAD comes in a child resistant package.
- Store MAVENCLAD at room temperature between 68°F and 77°F (20°C and 25°C).
- Store MAVENCLAD in the original package to protect from moisture.
- Ask your healthcare provider or pharmacist about how to safely throw away any unused or expired MAVENCLAD tablets and packaging.

Keep MAVENCLAD and all medicines out of the reach of children.

General information about the safe and effective use of MAVENCLAD

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide. Do not use MAVENCLAD for a condition for which it was not prescribed. Do not give MAVENCLAD to other people, even if they have the same symptoms that you have. It may harm them. You can ask your healthcare provider for information about MAVENCLAD that is written for health professionals.

What are the ingredients in MAVENCLAD?

Active ingredient: cladribine

Inactive ingredients: hydroxypropyl betadex, magnesium stearate, and sorbitol.

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MAVENCLAD is a registered trademark of Merck KGaA, Darmstadt, Germany.
For more information, call toll-free1-877-447-3243 or go to www.mavenclad.com
This Medication Guide has been approved by the U.S. Food and Drug Administration. Issued: 3/2019

EXHIBIT I

Conclusion:

Based on the results, the optimized complexation process was applied for the manufacture of the cladribine / hydroxypropyl betadex complex for Phase I clinical PK studies [redacted] the complex formed at the ratio of 1:14 w/w was selected for the manufacture of a 10 mg cladribine tablet for further Phase I/II/III clinical trials as well as for the commercial formulation.

1.3.4 Characterization of the Cladribine / Hydroxypropyl Betadex Complex

Characterization studies as follows were carried out on the complex:

- Powder X-Ray Diffraction (PXRD) and Differential Scanning Calorimetry (DSC)
- Solid-state Cross Polarization Magic Angle Spinning (CP-MAS) Nuclear Magnetic Resonance (NMR) Spectroscopy
- High Resolution 2D-NMR Spectroscopy
- Properties in Solution

1.3.4.1 Powder X-Ray Diffraction (PXRD) and Differential Scanning Calorimetry (DSC)

Lyophilized cladribine / hydroxypropyl betadex complex samples were investigated at the ratio 1:14 w/w by powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) for the identification of the physical form. For comparison purposes, cladribine and hydroxypropyl betadex lyophilized samples were also analyzed. For assessment of detection limits of potentially free (uncomplexed) cladribine, samples of lyophilized complex spiked with defined aliquots of crystalline cladribine and with defined aliquots of amorphous cladribine were prepared and also studied by PXRD and DSC.

[redacted]

[redacted]

Conclusion:

PXRD demonstrated that amorphous phase is obtained from the lyophilization process. Free crystalline cladribine is not detected in PXRD with limit of detection of free crystalline cladribine established at [redacted]%.

DSC demonstrated that the amorphous phase comprises a complex of cladribine and hydroxypropyl betadex.

1.3.4.2 Solid-state Cross Polarization Magic Angle Spinning (CP-MAS) Nuclear Magnetic Resonance (NMR) Spectroscopy

The complex of cladribine and hydroxypropyl betadex was investigated by 1D and 2D NMR methods. In a first step, the ^{13}C-cross polarized NMR spectra (^{13}C-CP NMR) of cladribine (both crystalline and amorphous form), hydroxypropyl betadex and the complex were recorded. [redacted]

[redacted]

Conclusion:

The results imply a different chemical environment for the cladribine molecules in the hydroxypropyl betadex complex, most likely driven by an intermolecular interaction.

1.3.4.3 High Resolution 2D NMR Spectroscopy

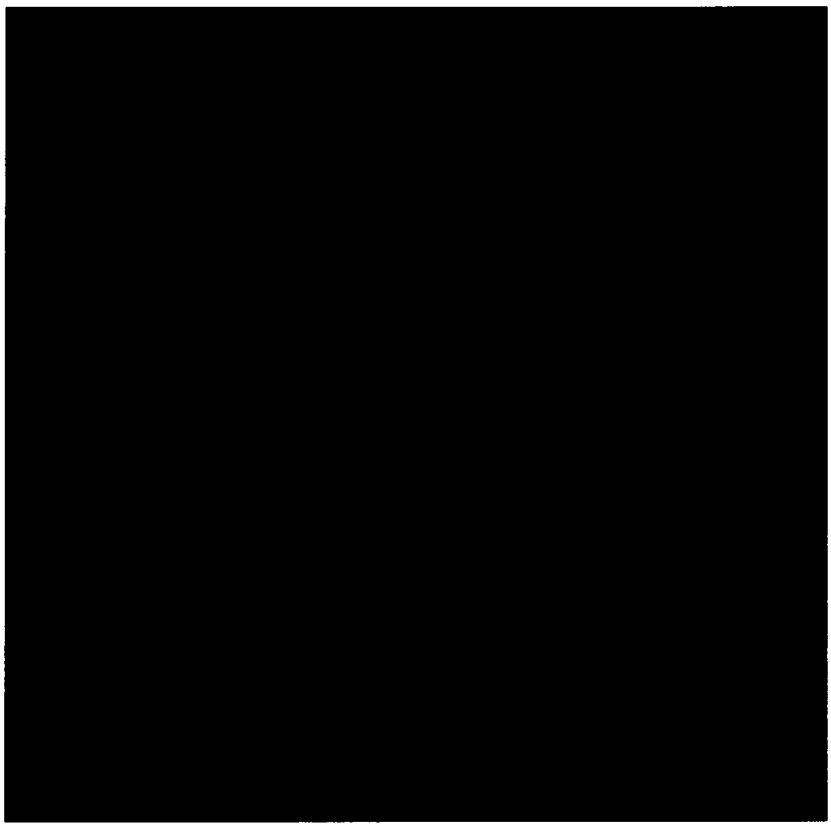
High resolution 2D NMR experiments were carried out in order to better understand the molecular interaction in the cladribine / hydroxypropyl betadex complex, namely TOCSY and ROESY (2).

[redacted]

[redacted]

[redacted]

[redacted]



[redacted]

Conclusion:

The cladribine / hydroxypropyl betadex complex is based on non-covalent interaction proving close spatial proximity between cladribine and the anomeric hydrogen on the hydroxypropyl betadex.

The 2D NMR data suggest formation of an inclusion complex.

1.3.4.4 Properties in Solution

Solubility studies were performed on free cladribine and complexed cladribine with hydroxypropyl betadex at different pH values [redacted]. Solubility of cladribine is enhanced when complexed. In addition, the studies show that hydrolysis rate of cladribine in acidic solution is significantly reduced for complexed cladribine compared to free cladribine and, hence, substantiate the interaction of cladribine with hydroxypropyl betadex [redacted]

Conclusion:

The results of the solubility studies demonstrate that cladribine shows an enhanced solubility and is stabilized in acidic solution when complexed by hydroxypropyl betadex. The formation of the impurity [redacted] is delayed by the complex formation.

[redacted]

1.4 Formulations used for Phase I Pharmacokinetic Studies

The [redacted] formulations developed, namely cladribine / cyclodextrin complex tablets ([redacted] mg and 10 mg), [redacted] The compositions of the [redacted] oral formulations used in the Phase I studies are presented in the tables below (see Table 6 through [redacted]).

Table 6 Composition of Cladribine / Hydroxypropyl Betadex Complex Tablet [redacted] mg (Batch N0120)

[redacted]

Table 7 **Composition of Cladribine / Hydroxypropyl Betadex Complex Tablet 10.0 mg (Batch N0126)**

Component	Formula (mg / Tablet)	Function
Cladribine	10.00	Active ingredient
Hydroxypropyl betadex	143.75	Solubility enhancer Stabilizing agent
Sorbitol	[redacted]	
Magnesium stearate		
Total Tablet Weight		-

[redacted]

1.5 Formulation Selected for Further Phase I/II/III Clinical Trials

Based on the results of the Phase I studies [redacted], the 10 mg cladribine / hydroxypropyl betadex complex tablet was selected for further Phase I/II/III clinical trials. The composition of the tablets applied for the Phase I/II/III clinical trials was identical to the 10 mg cladribine cyclodextrin tablet used for studies [redacted].

[redacted]

The Phase I/II/III clinical studies performed with the application of the 10 mg cladribine tablets are listed in Table 10.

Table 10 Cladribine Tablet Batches Used in Further Phase I/II/III Clinical Studies

Clinical Phase	Study Name	Clinical Batch No.
I	[redacted]	[redacted]
I	[redacted]	[redacted]
II	[redacted]	[redacted]
III	CLARITY – Efficacy & safety v. placebo, pivotal	[redacted]
III	[redacted]	[redacted]
III	[redacted]	[redacted]
III	CLARITY Extension – Safety, tolerability, and efficacy v. placebo	[redacted]
III	ORACLE – Efficacy and safety v. placebo	[redacted]

1.6 Commercial Formulation

To sustain a blend with [redacted], minor modifications of the formulation were made to obtain a process easily reproducible at a commercial scale. There was no change of the 14:1 w/w ratio between hydroxypropyl betadex and cladribine. [redacted]

[redacted]

[redacted]

Table 11 Composition of Cladribine 10 mg Tablets (Clinical versus Commercial Formulation)

Component	Clinical Formulation (mg / Tablet)	Commercial Formulation (mg / Tablet)	Function
Drug substance			
Cladribine	10.00	10.00	Active ingredient
Other ingredients			
Hydroxypropyl betadex (HPβCD)	143.76	143.76	Solubility enhancer Stabilizing agent
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
Total Tablet Weight	[redacted]	[redacted]	

For batch N0126, the amount of hydroxypropyl betadex was 143.75 mg [redacted]

In addition, the shape of the tablet was modified [redacted]

1.6.1 Comparability of Commercial and Clinical Formulations

Prior to recommending the use of the optimized formulation for commercial use, a detailed comparability exercise was performed. The quantitative composition of the "clinical" 10 mg cladribine / hydroxypropyl betadex tablets is comparable with that of the optimized "commercial" final product. [redacted]

EXHIBIT J

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

NDA 22561

NDA APPROVAL

EMD Serono, Inc.
Attention: Tammy Sarnelli, MPA
Head of Global Regulatory Affairs-Immunology and Neurology
45A Middlesex Turnpike
Billerica, MA 01821

Dear Ms. Sarnelli:

Please refer to your New Drug Application (NDA) dated May 30, 2018, received May 31, 2018, and your amendments, submitted under section 505(b)(1) Federal Food, Drug, and Cosmetic Act (FDCA) for Mavenclad (cladribine) tablets, 10 mg.

We also refer to your NDA originally submitted September 30, 2009; to our Complete Response letter dated February 28, 2011; and to your NDA withdrawal request dated August 19, 2011. Your NDA submission dated May 31, 2018, is considered a "resubmission after withdrawal" and responds to all of the items listed in our February 28, 2011, Complete Response letter.

This NDA provides for the use of Mavenclad (cladribine) tablets, 10 mg, for the treatment of relapsing forms of multiple sclerosis (MS), to include relapsing-remitting disease and active secondary progressive disease, in adults.

APPROVAL & LABELING

We have completed our review of this application, as amended. It is approved, effective on the date of this letter, for use as recommended in the enclosed agreed-upon labeling text.

CONTENT OF LABELING

As soon as possible, but no later than 14 days from the date of this letter, submit the content of labeling [21 CFR 314.50(l)] in structured product labeling (SPL) format using the FDA automated drug registration and listing system (eLIST), as described at http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm. Content of labeling must be identical to the enclosed labeling (text for the Prescribing Information and Medication Guide) as well as annual reportable changes not included in the enclosed labeling. Information on submitting SPL files using eLIST may be found in the guidance for industry *SPL Standard for Content of Labeling Technical Qs and As*, available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf

The SPL will be accessible via publicly available labeling repositories.

CARTON AND CONTAINER LABELING

Submit final printed carton and container labeling that are identical to the carton and container labeling submitted on January 30, 2019, as soon as they are available, but no more than 30 days after they are printed. Please submit these labeling electronically according to the guidance for industry titled *Providing Regulatory Submissions in Electronic Format — Certain Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (April 2018, Revision 5)*. For administrative purposes, designate this submission "**Final Printed Carton and Container Labeling for approved NDA 22561**." Approval of this submission by FDA is not required before the labeling is used.

REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients (which includes new salts and new fixed combinations), new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication in pediatric patients unless this requirement is waived, deferred, or inapplicable.

We are waiving the pediatric study requirement for this application because there is evidence strongly suggesting that the drug product would be unsafe in all pediatric age groups. The long-term risk of malignancies in adult subjects treated with cladribine is an unacceptable risk in pediatric patients.

POSTMARKETING REQUIREMENTS UNDER 505(o)

Section 505(o)(3) of the FDCA authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute.

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to assess a known serious risk of malignancy or assess a known serious risk of teratogenicity.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA will not be sufficient to assess these serious risks.

Therefore, based on appropriate scientific data, FDA has determined that you are required to conduct the following studies:

3592-1 Conduct an observational study to assess the long-term risk of malignancy for Mavenclad compared to other therapies used in the treatment of adults with relapsing forms of multiple sclerosis. Describe and justify the choice of appropriate comparator population(s) and estimated background rate(s) relative to

cladribine-exposed patients; clearly define the primary comparator population. Design the study around a testable hypothesis to assess, with sufficient sample size and power, a clinically meaningful increase in malignancy risk above the comparator background rate(s), with a pre-specified statistical analysis method. Specify concise case definitions and validation algorithms. For the Mavenclad-exposed and comparator(s) cohorts, clearly define the study drug initiation period and any exclusion and inclusion criteria. Enroll patients over an initial 4-year period and follow for a minimum of 8 years from the time of enrollment.

The timetable you submitted on March 19, 2019, states that you will conduct this study according to the following schedule:

Draft Protocol Submission:	08/2019
Final Protocol Submission:	09/2020
Study Completion:	02/2033
Final Report Submission:	02/2034

3592-2 Establish a worldwide Pregnancy Surveillance Program to collect and analyze information for a minimum of 10 years on pregnancy complications and birth outcomes in women exposed to Mavenclad during pregnancy. Provide a complete protocol which includes details regarding how you plan to encourage patients and providers to report pregnancy exposures (e.g., telephone contact number and/or website in prescribing information), measures to ensure complete data capture regarding pregnancy outcomes, and any adverse effects in offspring and plans for comprehensive data analysis and yearly reporting.

The timetable you submitted on March 21, 2019, states that you will conduct this study according to the following schedule:

Draft Protocol Submission:	08/2019
Final Protocol Submission:	09/2020
Annual Interim Report:	09/2021
	09/2022
	09/2023
	09/2024
	09/2025
	09/2026
	09/2027
	09/2028
	09/2029
	09/2030
Study Completion:	02/2031
Final Report Submission:	02/2032

Finally, we have determined that only a clinical trial (rather than a nonclinical or observational study) will be sufficient to identify an unexpected serious risk of a drug-drug interaction between Mavenclad and oral contraceptives.

Therefore, based on appropriate scientific data, FDA has determined that you are required to conduct the following trials:

3592-3 Conduct a clinical drug-drug interaction trial to evaluate the effect of cladribine on the pharmacokinetics (PK) of oral contraceptives. Include an evaluation of the effect on the components ethinyl estradiol (EE) and norelgestromin (NGMN).

The timetable you submitted on March 19, 2019, states that you will conduct this trial according to the following schedule:

Draft Protocol Submission:	06/2019
Final Protocol Submission:	06/2020
Trial Completion:	08/2023
Final Report Submission:	08/2024

Submit clinical protocols to your IND 74634, with a cross-reference letter to this NDA. Submit nonclinical and chemistry, manufacturing, and controls protocols and all final reports to your NDA. Prominently identify the submission with the following wording in bold capital letters at the top of the first page of the submission, as appropriate: **Required Postmarketing Protocol Under 505(o), Required Postmarketing Final Report Under 505(o), Required Postmarketing Correspondence Under 505(o).**

Submission of the protocols for required postmarketing observational studies to your IND is for purposes of administrative tracking only. These studies do not constitute clinical investigations pursuant to 21 CFR 312.3(b) and therefore are not subject to the IND requirements under 21 CFR part 312 or FDA's regulations under 21 CFR parts 50 (Protection of Human Subjects) and 56 (Institutional Review Boards).

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 314.81(b)(2)(vii) requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 314.81(b)(2)(vii) to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 314.81(b)(2)(vii). We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required

under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

REQUESTED PHARMACOVIGILANCE

We request that you perform postmarketing surveillance for malignancies, opportunistic infections, graft-versus-host disease with blood transfusion, liver injury, serious skin reactions, and acute cardiac failure after exposure to Mavenclad. We request that you provide expedited reports directly to the Division of Neurology Products. Include comprehensive summaries and analyses of these events quarterly as part of your required postmarketing safety reports [e.g., periodic safety update reports (PSURs)].

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the Prescribing Information, Medication Guide, and Patient Package Insert (as applicable) to:

OPDP Regulatory Project Manager
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Prescription Drug Promotion
5901-B Ammendale Road
Beltsville, MD 20705-1266

Alternatively, you may submit a request for advisory comments electronically in eCTD format. For more information about submitting promotional materials in eCTD format, see the draft Guidance for Industry (available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM443702.pdf).

As required under 21 CFR 314.81(b)(3)(i), you must submit final promotional materials, and the Prescribing Information, at the time of initial dissemination or publication, accompanied by a Form FDA 2253. Form FDA 2253 is available at http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM083570.pdf. Information and Instructions for completing the form can be found at http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM375154.pdf. For more information about submission of promotional materials to the Office of Prescription Drug Promotion (OPDP), see http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm.

REPORTING REQUIREMENTS

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

If you have any questions, please call Sandra Folkendt, Senior Regulatory Project Manager, at (240) 402-2804.

Sincerely,

{See appended electronic signature page}

Eric Bastings, MD
Deputy Director
Division of Neurology Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

ENCLOSURE(S):
Content of Labeling
Prescribing Information
Medication Guide

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

ERIC P BASTINGS
03/29/2019 04:27:10 PM

EXHIBIT K

[redacted]

From: Folkendt, Sandra <Sandra.Folkendt@fda.hhs.gov>
Sent: Friday, March 29, 2019 4:42 PM
To: Tammy Sarnelli <tammy.sarnelli@emdserono.com>
Subject: NDA 22561 Action Letter

Hello Tammy,

Please find attached a courtesy copy of the action letter for NDA 22561. A hard copy will be provided by mail. Kindly confirm receipt of this message.

Have a nice weekend!
Sandy

Sandra Folkendt
Regulatory Health Project Manager

Center of Drug Evaluation and Research
Division of Neurology Products
U.S. Food and Drug Administration
Tel: 240 402-2804
Sandra.Folkendt@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

EXHIBIT L

Drugs@FDA: FDA Approved Drug Products

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New Drug Application (NDA): 020229
Company: JANSSEN PHARMS

Products on NDA 020229

CSV | Excel | Print

Drug Name	Active Ingredients	Strength	Dosage Form/Route	Marketing Status	TE Code	RLD	RS
LEUSTATIN	CLADRIBINE	1MG/ML **Federal Register determination that product was not discontinued or withdrawn for safety or efficacy reasons**	INJECTABLE;INJECTION	Discontinued	None	Yes	No

Showing 1 to 1 of 1 entries

Approval Date(s) and History, Letters, Labels, Reviews for NDA 020229

Original Approvals or Tentative Approvals

CSV Excel Print

Action Date	Submission	Action Type	Submission Classification	Review Priority; Orphan Status	Letters, Reviews, Labels, Patient Package Insert	Notes
02/26/1993	ORIG-1	Approval	Type 1 - New Molecular Entity	STANDARD		Withdrawn FR Effective 11/03/2016 Label is not available on this site.

Showing 1 to 1 of 1 entries

Supplements

CSV Excel Print

Action Date	Submission	Supplement Categories or Approval Type	Letters, Reviews, Labels, Patient Package Insert
08/02/2012	SUPPL-34		**Label (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/020 Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2012/**
06/29/2006	SUPPL-30		**Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2006/**

Action Date	Submission	Supplement Categories or Approval Type	Letters, Reviews, Labels, Patient Package Insert
08/22/2002	SUPPL-21		Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2002/
08/20/2002	SUPPL-7		Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2002/
08/20/2002	SUPPL-4		Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2002/

Showing 1 to 5 of 5 entries

Labels for NDA 020229

LEUSTATIN® (cladribine)
Injection
For Intravenous Infusion Only

WARNING

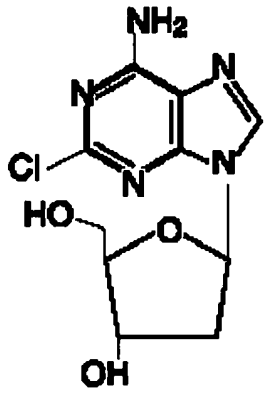
LEUSTATIN (cladribine) Injection should be administered under the supervision of a qualified physician experienced in the use of antineoplastic therapy. Suppression of bone marrow function should be anticipated. This is usually reversible and appears to be dose dependent. Serious neurological toxicity (including irreversible paraparesis and quadraparesis) has been reported in patients who received LEUSTATIN Injection by continuous infusion at high doses (4 to 9 times the recommended dose for Hairy Cell Leukemia). Neurologic toxicity appears to demonstrate a dose relationship; however, severe neurological toxicity has been reported rarely following treatment with standard cladribine dosing regimens.

Acute nephrotoxicity has been observed with high doses of LEUSTATIN (4 to 9 times the recommended dose for Hairy Cell Leukemia), especially when given concomitantly with other nephrotoxic agents/therapies.

DESCRIPTION

LEUSTATIN (cladribine) Injection (also commonly known as 2-chloro-2′-deoxy-β-D-adenosine) is a synthetic antineoplastic agent for continuous intravenous infusion. It is a clear, colorless, sterile, preservative-free, isotonic solution. LEUSTATIN Injection is available in single-use vials containing 10 mg (1 mg/mL) of cladribine, a chlorinated purine nucleoside analog. Each milliliter of LEUSTATIN Injection contains 1 mg of the active ingredient and 9 mg (0.15 mEq) of sodium chloride as an inactive ingredient. The solution has a pH range of 5.5 to 8.0. Phosphoric acid and/or dibasic sodium phosphate may have been added to adjust the pH to 6.3±0.3.

The chemical name for cladribine is 2-chloro-6-amino-9-(2-deoxy-β-D-erythropento-furanosyl) purine and the structure is represented below:



cladribine MW 285.7

CLINICAL PHARMACOLOGY

Cellular Resistance and Sensitivity:

The selective toxicity of 2-chloro-2′-deoxy-β-D-adenosine towards certain normal and malignant lymphocyte and monocyte populations is based on the relative activities of deoxycytidine kinase and deoxynucleotidase. Cladribine passively crosses the cell membrane. In cells with a high ratio of deoxycytidine kinase to deoxynucleotidase, it is phosphorylated by deoxycytidine kinase to 2-chloro-2′-deoxy- β -D-adenosine monophosphate (2-CdAMP). Since 2-chloro-2′-deoxy- β -D-adenosine is resistant to deamination by adenosine deaminase and there is little deoxynucleotide deaminase in lymphocytes and monocytes, 2-CdAMP accumulates intracellularly and is subsequently converted into the active triphosphate deoxynucleotide, 2-chloro-2′-deoxy- β -D-adenosine triphosphate (2-CdATP). It is postulated that cells with high deoxycytidine kinase and low deoxynucleotidase activities will be selectively killed by 2-chloro-2′-deoxy- β -D-adenosine as toxic deoxynucleotides accumulate intracellularly.

Cells containing high concentrations of deoxynucleotides are unable to properly repair single-strand DNA breaks. The broken ends of DNA activate the enzyme poly (ADP-ribose) polymerase resulting in NAD and ATP depletion and disruption of cellular metabolism. There is evidence, also, that 2-CdATP is incorporated into the DNA of dividing cells, resulting in impairment of DNA synthesis. Thus, 2-chloro-2′-deoxy- β -D-adenosine can be distinguished from other chemotherapeutic agents affecting purine metabolism in that it is cytotoxic to both actively dividing and quiescent lymphocytes and monocytes, inhibiting both DNA synthesis and repair.

Pharmacokinetics

In a clinical investigation, 17 patients with Hairy Cell Leukemia and normal renal function were treated for 7 days with the recommended treatment regimen of LEUSTATIN Injection (0.09 mg/kg/day) by continuous intravenous infusion. The mean steady-state serum concentration was estimated to be 5.7 ng/mL with an estimated systemic clearance of 663.5 mL/h/kg when LEUSTATIN was given by continuous infusion over 7 days. In Hairy Cell Leukemia patients, there does not appear to be a relationship between serum concentrations and ultimate clinical outcome.

In another study, 8 patients with hematologic malignancies received a two (2) hour infusion of LEUSTATIN Injection (0.12 mg/kg). The mean end-of-infusion plasma LEUSTATIN concentration was 48±19 ng/mL. For 5 of these patients, the disappearance of LEUSTATIN could be described by either a biphasic or triphasic decline. For these patients with normal renal function, the mean terminal half-life was 5.4 hours. Mean values for clearance and steady-state volume of distribution were 978±422 mL/h/kg and 4.5±2.8 L/kg, respectively.

Cladribine plasma concentration after intravenous administration declines multi-exponentially with an average half-life of 6.7 +/- 2.5 hours. In general, the apparent volume of distribution of cladribine is approximately 9 L/kg, indicating an extensive distribution in body tissues.

Cladribine penetrates into cerebrospinal fluid. One report indicates that concentrations are approximately 25% of those in plasma.

LEUSTATIN is bound approximately 20% to plasma proteins.

Except for some understanding of the mechanism of cellular toxicity, no other information is available on the metabolism of LEUSTATIN in humans. An average of 18% of the administered dose has been reported to be excreted in urine of patients with solid tumors during a 5-day continuous intravenous infusion of 3.5-8.1 mg/m^2/day of LEUSTATIN. The effect of renal and hepatic impairment on the elimination of cladribine has not been investigated in humans.

CLINICAL STUDIES

Two single-center open label studies of LEUSTATIN (cladribine) have been conducted in patients with Hairy Cell Leukemia with evidence of active disease requiring therapy. In the study conducted at the Scripps Clinic and Research Foundation (Study A), 89 patients were treated with a single course of LEUSTATIN Injection given by continuous intravenous infusion for 7 days at a dose of

0.09 mg/kg/day. In the study conducted at the M.D. Anderson Cancer Center (Study B), 35 patients were treated with a 7-day continuous intravenous infusion of LEUSTATIN Injection at a comparable dose of 3.6 mg/m^2/day. A complete response (CR) required clearing of the peripheral blood and bone marrow of hairy cells and recovery of the hemoglobin to 12 g/dL, platelet count to 100 x 10^9/L, and absolute neutrophil count to 1500 x 10^6/L. A good partial response (GPR) required the same hematologic parameters as a complete response, and that fewer than 5% hairy cells remain in the bone marrow. A partial response (PR) required that hairy cells in the bone marrow be decreased by at least 50% from baseline and the same response for hematologic parameters as for complete response. A pathologic relapse was defined as an increase in bone marrow hairy cells to 25% of pretreatment levels. A clinical relapse was defined as the recurrence of cytopenias, specifically, decreases in hemoglobin $\geq$ 2 g/dL, ANC $\geq$ 25% or platelet counts $\geq$ 50,000. Patients who met the criteria for a complete response but subsequently were found to have evidence of bone marrow hairy cells (< 25% of pretreatment levels) were reclassified as partial responses and were not considered to be complete responses with relapse.

Among patients evaluable for efficacy (N=106), using the hematologic and bone marrow response criteria described above, the complete response rates in patients treated with LEUSTATIN Injection were 65% and 68% for Study A and Study B, respectively, yielding a combined complete response rate of 66%. Overall response rates (i.e., Complete plus Good Partial plus Partial Responses) were 89% and 86% in Study A and Study B, respectively, for a combined overall response rate of 88% in evaluable patients treated with LEUSTATIN Injection.

Using an intent-to-treat analysis (N=123) and further requiring no evidence of splenomegaly as a criterion for CR (i.e., no palpable spleen on physical examination and $\leq$ 13 cm on CT scan), the complete response rates for Study A and Study B were 54% and 53%, respectively, giving a combined CR rate of 54%. The overall response rates (CR + GPR + PR) were 90% and 85%, for Studies A and B, respectively, yielding a combined overall response rate of 89%.

RESPONSE RATES TO LEUSTATIN TREATMENT IN PATIENTS WITH HAIRY CELL LEUKEMIA

	CR	Overall
Evaluable Patients N=106	66%	88%
Intent-to-treat Population N=123	54%	89%

In these studies, 60% of the patients had not received prior chemotherapy for Hairy Cell Leukemia or had undergone splenectomy as the only prior treatment and were receiving

LEUSTATIN as a first-line treatment. The remaining 40% of the patients received LEUSTATIN as a second-line treatment, having been treated previously with other agents, including α-interferon and/or deoxycoformycin. The overall response rate for patients without prior chemotherapy was 92%, compared with 84% for previously treated patients. LEUSTATIN is active in previously treated patients; however, retrospective analysis suggests that the overall response rate is decreased in patients previously treated with splenectomy or deoxycoformycin and in patients refractory to α-interferon.

OVERALL RESPONSE RATES (CR + GPR + PR) TO LEUSTATIN TREATMENT IN PATIENTS WITH HAIRY CELL LEUKEMIA

	OVERALL RESPONSE (N = 123)	NR + RELAPSE
No Prior Chemotherapy	68/74	6 + 4
	92%	14%
Any Prior Chemotherapy	41/49	8 + 3
	84%	22%
Previous Splenectomy	32/41*	9 + 1
	78%	24%
Previous Interferon	40/48	8 + 3
	83%	23%
Interferon Refractory	6/11*	5 + 2
	55%	64%
Previous Deoxycoformycin	3/6*	3 + 1
	50%	66%

NR = No Response
* $P < 0.05$

After a reversible decline, normalization of peripheral blood counts (Hemoglobin >12.0 g/dL, Platelets >100 x 10^9/L, Absolute Neutrophil Count (ANC) >1500 x 10^6/L) was achieved by 92% of evaluable patients. The median time to normalization of peripheral counts was 9 weeks from the start of treatment (Range: 2 to 72). The median time to normalization of Platelet Count was 2 weeks, the median time to normalization of ANC was 5 weeks and the median time to normalization of Hemoglobin was 8 weeks. With normalization of Platelet Count and Hemoglobin, requirements for platelet and RBC transfusions were abolished after Months 1 and 2, respectively, in those patients with complete response. Platelet recovery may be delayed in a minority of patients with severe baseline thrombocytopenia. Corresponding to normalization of ANC, a trend toward a reduced incidence of infection was seen after the third month, when compared to the months immediately preceding LEUSTATIN therapy. (see also WARNINGS, PRECAUTIONS and ADVERSE REACTIONS)

LEUSTATIN TREATMENT IN PATIENTS WITH HAIRY CELL LEUKEMIA TIME TO NORMALIZATION OF PERIPHERAL BLOOD COUNTS

Parameter	Median Time to Normalization of Count*
Platelet Count	2 weeks
Absolute Neutrophil Count	5 weeks
Hemoglobin	8 weeks
ANC, Hemoglobin and Platelet Count	9 weeks

* Day 1 = First day of infusion

For patients achieving a complete response, the median time to response (i.e., absence of hairy cells in bone marrow and peripheral blood together with normalization of peripheral blood parameters), measured from treatment start, was approximately 4 months. Since bone marrow aspiration and biopsy were frequently not performed at the time of peripheral blood normalization, the median time to complete response may actually be shorter than that which was recorded. At the time of data cut-off, the median duration of complete response was greater than 8 months and ranged to 25+ months. Among 93 responding patients, seven had shown evidence of disease progression at the time of the data cut-off. In four of these patients, disease was limited to the bone marrow without peripheral blood abnormalities (pathologic progression), while in three patients there were also peripheral blood abnormalities (clinical progression). Seven patients who did not respond to a first course of LEUSTATIN received a second course of therapy. In the five patients who had adequate follow-up, additional courses did not appear to improve their overall response.

INDICATIONS FOR USE

LEUSTATIN Injection is indicated for the treatment of active Hairy Cell Leukemia as defined by clinically significant anemia, neutropenia, thrombocytopenia or disease-related symptoms.

CONTRAINDICATIONS

LEUSTATIN Injection is contraindicated in those patients who are hypersensitive to this drug or any of its components.

WARNINGS

Due to increased risk of infection in the setting of immunosuppression with chemotherapy including LEUSTATIN, it is recommended not to administer live attenuated vaccines to patients receiving LEUSTATIN Injection.

Severe bone marrow suppression, including neutropenia, anemia and thrombocytopenia, has been commonly observed in patients treated with LEUSTATIN, especially at high doses. At initiation of treatment, most patients in the clinical studies had hematologic impairment as a manifestation of active Hairy Cell

Leukemia. Following treatment with LEUSTATIN, further hematologic impairment occurred before recovery of peripheral blood counts began. During the first two weeks after treatment initiation, mean Platelet Count, ANC, and Hemoglobin concentration declined and subsequently increased with normalization of mean counts by Day 12, Week 5 and Week 8, respectively. The myelosuppressive effects of LEUSTATIN were most notable during the first month following treatment. Forty-four percent (44%) of patients received transfusions with RBCs and 14% received transfusions with platelets during Month 1. Careful hematologic monitoring, especially during the first 4 to 8 weeks after treatment with LEUSTATIN Injection, is recommended (see PRECAUTIONS).

Fever ($T \geq 100°F$) was associated with the use of LEUSTATIN in approximately two-thirds of patients (131/196) in the first month of therapy. Virtually all of these patients were treated empirically with parenteral antibiotics. Overall, 47% (93/196) of all patients had fever in the setting of neutropenia ($ANC \leq 1000$), including 62 patients (32%) with severe neutropenia (i.e., $ANC \leq 500$).

In a Phase I investigational study using LEUSTATIN in high doses (4 to 9 times the recommended dose for Hairy Cell Leukemia) as part of a bone marrow transplant conditioning regimen, which also included high dose cyclophosphamide and total body irradiation, acute nephrotoxicity and delayed onset neurotoxicity were observed. Thirty-one (31) poor-risk patients with drug-resistant acute leukemia in relapse (29 cases) or non-Hodgkins Lymphoma (2 cases) received LEUSTATIN for 7 to 14 days prior to bone marrow transplantation. During infusion, 8 patients experienced gastrointestinal symptoms. While the bone marrow was initially cleared of all hematopoietic elements, including tumor cells, leukemia eventually recurred in all treated patients. Within 7 to 13 days after starting treatment with LEUSTATIN, 6 patients (19%) developed manifestations of renal dysfunction (e.g., acidosis, anuria, elevated serum creatinine, etc.) and 5 required dialysis. Several of these patients were also being treated with other medications having known nephrotoxic potential. Renal dysfunction was reversible in 2 of these patients. In the 4 patients whose renal function had not recovered at the time of death, autopsies were performed; in 2 of these, evidence of tubular damage was noted. Eleven (11) patients (35%) experienced delayed onset neurologic toxicity. In the majority, this was characterized by progressive irreversible motor weakness (paraparesis/quadriparesis) of the upper and/or lower extremities, first noted 35 to 84 days after starting high dose therapy with LEUSTATIN. Non-invasive testing (electromyography and nerve conduction studies) was consistent with demyelinating disease. Severe neurologic toxicity has also been noted with high doses of another drug in this class.

Axonal peripheral polyneuropathy was observed in a dose escalation study at the highest dose levels (approximately 4 times the recommended dose for Hairy Cell Leukemia) in patients not receiving cyclophosphamide or total body irradiation. Severe neurological toxicity has been reported rarely following treatment with standard cladribine dosing regimens.

In patients with Hairy Cell Leukemia treated with the recommended treatment regimen (0.09 mg/kg/day for 7 consecutive days), there have been no reports of nephrologic toxicities.

Serious (e.g. respiratory infection, pneumonia and viral skin infections), including fatal infections (e.g. sepsis) were reported (see ADVERSE REACTIONS).

Of the 196 Hairy Cell Leukemia patients entered in the two trials, there were 8 deaths following treatment. Of these, 6 were of infectious etiology, including 3 pneumonias, and 2 occurred in the first month following LEUSTATIN therapy. Of the 8 deaths, 6 occurred in previously treated patients who were refractory to α interferon.

Benzyl alcohol is a constituent of the recommended diluent for the 7-day infusion solution. Benzyl alcohol has been reported to be associated with a fatal "Gasping Syndrome" in premature infants. (see DOSAGE AND ADMINISTRATION)

Pregnancy Category D:

LEUSTATIN can cause fetal harm when administered to a pregnant woman. Although there is no evidence of teratogenicity in humans due to LEUSTATIN, other drugs which inhibit DNA synthesis have been reported to be teratogenic in humans. Cladribine is teratogenic in animals. Advise females of reproductive potential to use highly effective contraception during treatment with LEUSTATIN. If LEUSTATIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

Cladribine is teratogenic in mice and rabbits and consequently has the potential to cause fetal harm when administered to a pregnant woman. A significant increase in fetal variations was observed in mice receiving 1.5 mg/kg/day (4.5 mg/m^2) and increased resorptions, reduced litter size and increased fetal malformations were observed when mice received 3.0 mg/kg/day (9 mg/m^2). Fetal death and malformations were observed in rabbits that received 3.0 mg/kg/day (33.0 mg/m^2). No fetal effects were seen in mice at 0.5 mg/kg/day (1.5 mg/m^2) or in rabbits at 1.0 mg/kg/day (11.0 mg/m^2).

PRECAUTIONS

General:

LEUSTATIN Injection is a potent antineoplastic agent with potentially significant toxic side effects. It should be administered only under the supervision of a physician experienced with the use of cancer chemotherapeutic agents. Patients undergoing therapy should be closely observed for signs of hematologic and non-hematologic toxicity. Periodic assessment of peripheral blood counts, particularly during the first 4 to 8 weeks post-treatment, is recommended to detect the development of anemia, neutropenia and thrombocytopenia and for early detection of any potential sequelae (e.g., infection or bleeding). As with other potent chemotherapeutic agents, monitoring of renal and hepatic function is also recommended, especially in patients with underlying kidney or liver dysfunction (see WARNINGS and ADVERSE REACTIONS).

Fever was a frequently observed side effect during the first month on study. Since the majority of fevers occurred in neutropenic patients, patients should be closely monitored during the first month of treatment and empiric antibiotics should be initiated as clinically indicated. Although 69% of patients developed fevers, less than 1/3 of febrile events were associated with documented infection. Given the known myelosuppressive effects of LEUSTATIN, practitioners should carefully evaluate the risks and benefits of administering this drug to patients with active infections (see WARNINGS and ADVERSE REACTIONS).

There are inadequate data on dosing of patients with renal or hepatic insufficiency. Development of acute renal insufficiency in some patients receiving high doses of LEUSTATIN has been described. Until more information is available, caution is advised when administering the drug to patients with known or suspected renal or hepatic insufficiency (see WARNINGS).

Rare cases of tumor lysis syndrome have been reported in patients treated with cladribine with other hematologic malignancies having a high tumor burden.

LEUSTATIN Injection must be diluted in designated intravenous solutions prior to administration (see DOSAGE AND ADMINISTRATION).

Laboratory Tests:

During and following treatment, the patient's hematologic profile should be monitored regularly to determine the degree of hematopoietic suppression. In the clinical studies, following reversible declines in all cell counts, the mean Platelet Count reached 100 x 10^9/L by Day 12, the mean Absolute Neutrophil Count reached 1500 x 10^6/L by Week 5 and the mean Hemoglobin reached 12 g/dL by Week 8.

After peripheral counts have normalized, bone marrow aspiration and biopsy should be performed to confirm response to treatment with LEUSTATIN. Febrile events should be investigated with appropriate laboratory and radiologic studies. Periodic assessment of renal function and hepatic function should be performed as clinically indicated.

Drug Interactions:

There are no known drug interactions with LEUSTATIN Injection. Caution should be exercised if LEUSTATIN Injection is administered before, after, or in conjunction with other drugs known to cause immunosuppression or myelosuppression. (see WARNINGS)

Carcinogenesis:

No animal carcinogenicity studies have been conducted with cladribine. However, its carcinogenic potential cannot be excluded based on demonstrated genotoxicity of cladribine.

Mutagenesis:

As expected for compounds in this class, the actions of cladribine yield DNA damage. In mammalian cells in culture, cladribine caused the accumulation of DNA strand breaks. Cladribine was also incorporated into DNA of human lymphoblastic leukemia cells. Cladribine was not mutagenic *in vitro* (Ames and Chinese hamster ovary cell gene mutation tests) and did not induce unscheduled DNA synthesis in primary rat hepatocyte cultures. However, cladribine was clastogenic both *in vitro* (chromosome aberrations in Chinese hamster ovary cells) and *in vivo* (mouse bone marrow micronucleus test).

Impairment of Fertility:

The effect on human fertility is unknown. When administered intravenously to Cynomolgus monkeys, cladribine has been shown to cause suppression of rapidly generating cells, including testicular cells.

Pregnancy:

Pregnancy Category D: (see WARNINGS).

Nursing Mothers:

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from cladribine, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug for the mother.

Pediatric Use:

Safety and effectiveness in pediatric patients have not been established. In a Phase I study involving patients 1-21 years old with relapsed acute leukemia, LEUSTATIN was given by continuous intravenous infusion in doses ranging from 3 to 10.7 mg/m^2/day for 5 days (one-half to twice the dose recommended in Hairy Cell Leukemia). In this study, the dose-limiting toxicity was severe myelosuppression with profound neutropenia and thrombocytopenia. At the highest dose (10.7 mg/m^2/day), 3 of 7 patients developed irreversible myelosuppression and fatal systemic bacterial or fungal infections. No unique toxicities were noted in this study [(1)] (see WARNINGS and ADVERSE REACTIONS).

Geriatric Use

Clinical studies of LEUSTATIN did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients. In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy in elderly patients.

ADVERSE REACTIONS

Clinical Trials Experience

Adverse drug reactions reported by ≥ 1% of LEUSTATIN-treated patients with HCL noted in the HCL clinical dataset (studies K90-091 and L91-048, n=576) are shown in the table below.

Adverse Drug Reactions in ≥ 1% of Patients Treated With LEUSTATIN in HCL Clinical Trials	
System Organ Class Preferred Term	**LEUSTATIN (n=576)** **%**
Blood and Lymphatic System Disorder (see also sections WARNINGS and PRECAUTIONS)	
Anemia	1
Febrile neutropenia	8
Psychiatric Disorders	
Anxiety	1
Insomnia	3
Nervous System Disorders	
Dizziness	6
Headache	14
Cardiac Disorders	
Tachycardia	2
Respiratory, Thoracic and Mediastinal Disorders	
Breath sounds abnormal	4
Cough	7
Dyspnea*	5
Rales	1
Gastrointestinal Disorders	
Abdominal pain**	4

Constipation	4
Diarrhea	7
Flatulence	1
Nausea	22
Vomiting	9
Skin and Subcutaneous Tissue Disorders	
Ecchymosis	2
Hyperhidrosis	3
Petechiae	2
Pruritus	2
Rash***	16
Musculoskeletal, Connective Tissue, and Bone Disorders	
Arthralgia	3
Myalgia	6
Pain****	6
General Disorders and Administration Site Conditions (see also sections WARNINGS and PRECAUTIONS)	
Administration site reaction*****	11
Asthenia	6
Chills	2
Decreased appetite	8
Fatigue	31
Malaise	5
Muscular weakness	1
Edema peripheral	2
Pyrexia	33
Injury, Poisoning and Procedural Complications	
Contusion	1

* Dyspnea includes dyspnea, dyspnea exertional, and wheezing
** Abdominal pain includes abdominal discomfort, abdominal pain, and abdominal pain (lower and upper)
*** Rash includes erythema, rash, and rash (macular, macula-papular, papular, pruritic, pustular and erythematous)
**** Pain includes pain, back pain, chest pain, arthritis pain, bone pain, and pain in extremity
***** Administration site reaction includes administration site reaction, catheter site (cellulitis, erythema, hemorrhage, and pain), and infusion site reaction(erythema, edema, and pain)

The following safety data are based on 196 patients with Hairy Cell Leukemia: the original cohort of 124 patients plus an additional 72 patients enrolled at the same two centers after the original enrollment cutoff. In Month 1 of the Hairy Cell Leukemia clinical trials, severe neutropenia was noted in 70% of patients, fever in 69%, and infection was documented in 28%. Most non-hematologic adverse experiences were mild to moderate in severity.

Myelosuppression was frequently observed during the first month after starting treatment. Neutropenia (ANC < 500 x 10^6/L) was noted in 70% of patients, compared with 26% in whom it was present initially. Severe anemia (Hemoglobin < 8.5 g/dL) developed in 37% of patients, compared with 10% initially and thrombocytopenia (Platelets < 20 x 10^9/L) developed in 12% of patients, compared to 4% in whom it was noted initially.

During the first month, 54 of 196 patients (28%) exhibited documented evidence of infection. Serious infections (e.g., septicemia, pneumonia) were reported in 6% of all

patients; the remainder were mild or moderate. Several deaths were attributable to infection and/or complications related to the underlying disease. During the second month, the overall rate of documented infection was 6%; these infections were mild to moderate and no severe systemic infections were seen. After the third month, the monthly incidence of infection was either less than or equal to that of the months immediately preceding LEUSTATIN therapy.

During the first month, 11% of patients experienced severe fever (i.e., ≥104°F). Documented infections were noted in fewer than one-third of febrile episodes. Of the 196 patients studied, 19 were noted to have a documented infection in the month prior to treatment. In the month following treatment, there were 54 episodes of documented infection: 23 (42%) were bacterial, 11 (20%) were viral and 11 (20%) were fungal. Seven (7) of 8 documented episodes of herpes zoster occurred during the month following treatment. Fourteen (14) of 16 episodes of documented fungal infections occurred in the first two months following treatment. Virtually all of these patients were treated empirically with antibiotics. (see WARNINGS and PRECAUTIONS)

Analysis of lymphocyte subsets indicates that treatment with cladribine is associated with prolonged depression of the CD4 counts. Prior to treatment, the mean CD4 count was 766/μL. The mean CD4 count nadir, which occurred 4 to 6 months following treatment, was 272/μL. Fifteen (15) months after treatment, mean CD4 counts remained below 500/μL. CD8 counts behaved similarly, though increasing counts were observed after 9 months. The clinical significance of the prolonged CD4 lymphopenia is unclear.

Another event of unknown clinical significance includes the observation of prolonged bone marrow hypocellularity. Bone marrow cellularity of < 35% was noted after 4 months in 42 of 124 patients (34%) treated in the two pivotal trials. This hypocellularity was noted as late as day 1010. It is not known whether the hypocellularity is the result of disease related marrow fibrosis or if it is the result of cladribine toxicity. There was no apparent clinical effect on the peripheral blood counts.

The vast majority of rashes were mild. Most episodes of nausea were mild, not accompanied by vomiting, and did not require treatment with antiemetics. In patients requiring antiemetics, nausea was easily controlled, most frequently with chlorpromazine.

When used in other clinical settings the following ADRs were reported: bacteremia, cellulitis, localized infection, pneumonia, anemia, thrombocytopenia (with bleeding or petechiae), phlebitis, purpura, crepitations, localized edema and edema.

For a description of adverse reactions associated with use of high doses in non-Hairy Cell Leukemia patients, see WARNINGS.

Postmarketing Experience

The following additional adverse reactions have been reported since the drug became commercially available. These adverse reactions have been reported primarily in patients who received multiple courses of LEUSTATIN Injection:

Infections and infestations: Septic shock. Opportunistic infections have occurred in the acute phase of treatment.

Blood and lymphatic system disorders: Bone marrow suppression with prolonged pancytopenia, including some reports of aplastic anemia; hemolytic anemia (including autoimmune hemolytic anemia), which was reported in patients with lymphoid malignancies, occurring within the first few weeks following treatment. Rare cases of myelodysplastic syndrome have been reported.

Immune system disorders: Hypersensitivity.

Metabolism and nutrition disorders: Tumor lysis syndrome.

Psychiatric disorders: Confusion (including disorientation).

Hepatobiliary disorders: Reversible, generally mild increases in bilirubin (uncommon) and transaminases.

Nervous System disorders: Depressed level of consciousness, neurological toxicity (including peripheral sensory neuropathy, motor neuropathy (paralysis), polyneuropathy, paraparesis); however, severe neurotoxicity has been reported rarely following treatment with standard cladribine dosing regimens.

Eye disorders: Conjunctivitis.

Respiratory, thoracic and mediastinal disorders: Pulmonary interstitial infiltrates (including lung infiltration, interstitial lung disease, pneumonitis and pulmonary fibrosis); in most cases, an infectious etiology was identified.

Skin and tissue disorders: Urticaria, hypereosinophilia; Stevens-Johnson. In isolated cases toxic epidermal necrolysis has been reported in patients who were receiving or had recently been treated with other medications (e.g., allopurinol or antibiotics) known to cause these syndromes.

Renal and urinary disorders: Renal failure (including renal failure acute, renal impairment).

OVERDOSAGE

High doses of LEUSTATIN have been associated with: irreversible neurologic toxicity (paraparesis/quadriparesis), acute nephrotoxicity, and severe bone marrow suppression resulting in neutropenia, anemia and thrombocytopenia (see WARNINGS). There is no known specific antidote to overdosage. Treatment of overdosage consists of discontinuation of LEUSTATIN, careful observation and appropriate supportive measures. It is not known whether the drug can be removed from the circulation by dialysis or hemofiltration.

DOSAGE AND ADMINISTRATION

Usual Dose:

The recommended dose and schedule of LEUSTATIN Injection for active Hairy Cell Leukemia is as a single course given by continuous infusion for 7 consecutive days at a dose of 0.09 mg/kg/day. Deviations from this dosage regimen are not advised. If the patient does not respond to the initial course of LEUSTATIN Injection for Hairy Cell Leukemia, it is unlikely that they will benefit from additional courses. Physicians should consider delaying or discontinuing the drug if neurotoxicity or renal toxicity occurs (see WARNINGS).

Specific risk factors predisposing to increased toxicity from LEUSTATIN have not been defined. In view of the known toxicities of agents of this class, it would be prudent to proceed carefully in patients with known or suspected renal insufficiency or severe bone marrow impairment of any etiology. Patients should be monitored closely for hematologic and non-hematologic toxicity (see WARNINGS and PRECAUTIONS).

Preparation and Administration of Intravenous Solutions:

LEUSTATIN Injection must be diluted with the designated diluent prior to administration. Since the drug product does not contain any anti-microbial preservative or bacteriostatic agent, **aseptic technique and proper environmental precautions must be observed in preparation of LEUSTATIN Injection solutions.**

To prepare a single daily dose:

LEUSTATIN Injection should be passed through a sterile 0.22μm disposable hydrophilic syringe filter prior to introduction into the infusion bag, prior to each daily infusion. Add the calculated dose (0.09 mg/kg or 0.09 mL/kg) of LEUSTATIN Injection through the sterile filter to an infusion bag containing 500 mL of 0.9% Sodium Chloride Injection, USP. Infuse continuously over 24 hours. Repeat daily for a total of 7 consecutive days. **The use of 5% dextrose as a diluent is not recommended because of increased degradation of cladribine.** Admixtures of

LEUSTATIN Injection are chemically and physically stable for at least 24 hours at room temperature under normal room fluorescent light in Baxter Viaflex®† PVC infusion containers. **Since limited compatibility data are available, adherence to the recommended diluents and infusion systems is advised.**

	Dose of LEUSTATIN Injection	Recommended Diluent	Quantity of Diluent
24-hour infusion method	1(day) x 0.09 mg/kg	0.9% Sodium Chloride Injection, USP	500 mL

To prepare a 7-day infusion:

The 7-day infusion solution should only be prepared with Bacteriostatic 0.9% Sodium Chloride Injection, USP (0.9% benzyl alcohol preserved). In order to minimize the risk of microbial contamination, both LEUSTATIN Injection and the diluent should be passed through a sterile 0.22 μm disposable hydrophilic syringe filter as each solution is being introduced into the infusion reservoir. First add the calculated dose of LEUSTATIN Injection (7 days x 0.09 mg/kg or mL/kg) to the infusion reservoir through the sterile filter.

Then add a calculated amount of Bacteriostatic 0.9% Sodium Chloride Injection, USP (0.9% benzyl alcohol preserved) also through the filter to bring the total volume of the solution to 100 mL. After completing solution preparation, clamp off the line, disconnect and discard the filter. Aseptically aspirate air bubbles from the reservoir as necessary using the syringe and a dry second sterile filter or a sterile vent filter assembly. Reclamp the line and discard the syringe and filter assembly. Infuse continuously over 7 days. Solutions prepared with Bacteriostatic Sodium Chloride Injection for individuals weighing more than 85 kg may have reduced preservative effectiveness due to greater dilution of the benzyl alcohol preservative. Admixtures for the 7-day infusion have demonstrated acceptable chemical and physical stability for at least 7 days in the SIMS Deltec MEDICATION CASSETTE™ Reservoir‡.

	Dose of LEUSTATIN Injection	Recommended Diluent	Quantity of Diluent
7-day infusion method (use sterile 0.22μ filter when preparing infusion solution)	7 (days) x 0.09 mg/kg	Bacteriostatic 0.9% Sodium Chloride Injection, USP (0.9% benzyl alcohol)	q.s. to 100 mL

Since limited compatibility data are available, adherence to the recommended diluents and infusion systems is advised. Solutions containing LEUSTATIN Injection should not be mixed with other intravenous drugs or additives or infused

simultaneously via a common intravenous line, since compatibility testing has not been performed. Preparations containing benzyl alcohol should not be used in neonates (see WARNINGS).

Care must be taken to assure the sterility of prepared solutions. Once diluted, solutions of LEUSTATIN Injection should be administered promptly or stored in the refrigerator (2° to 8° C) for no more than 8 hours prior to start of administration. Vials of LEUSTATIN Injection are for single-use only. Any unused portion should be discarded in an appropriate manner (see Handling and Disposal).

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. A precipitate may occur during the exposure of LEUSTATIN Injection to low temperatures; it may be resolubilized by allowing the solution to warm naturally to room temperature and by shaking vigorously. **DO NOT HEAT OR MICROWAVE.**

Chemical Stability of Vials:

When stored in refrigerated conditions between 2° to 8°C (36° to 46°F) protected from light, unopened vials of LEUSTATIN Injection are stable until the expiration date indicated on the package. Freezing does not adversely affect the solution. If freezing occurs, thaw naturally to room temperature. DO NOT heat or microwave. Once thawed, the vial of LEUSTATIN Injection is stable until expiry if refrigerated. DO NOT refreeze. Once diluted, solutions containing LEUSTATIN Injection should be administered promptly or stored in the refrigerator (2° to 8°C) for no more than 8 hours prior to administration.

Handling and Disposal:

The potential hazards associated with cytotoxic agents are well established and proper precautions should be taken when handling, preparing, and administering LEUSTATIN Injection. The use of disposable gloves and protective garments is recommended. If LEUSTATIN Injection contacts the skin or mucous membranes, wash the involved surface immediately with copious amounts of water. Several guidelines on this subject have been published.[2-8] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate. Refer to your Institution's guidelines and all applicable state/local regulations for disposal of cytotoxic waste.

HOW SUPPLIED

LEUSTATIN Injection is supplied as a sterile, preservative-free, isotonic solution containing 10 mg (1 mg/mL) of cladribine as 10 mL filled into a single-use clear flint

glass 20 mL vial. LEUSTATIN Injection is supplied in 10 mL (1 mg/mL) single-use vials (NDC 59676-201-01) available in a treatment set (case) of seven vials.

Store refrigerated 2° to 8°C (36° to 46°F). Protect from light during storage.

REFERENCES:

1. Santana VM, Mirro J, Harwood FC, *et al*: A phase I clinical trial of 2-Chloro-deoxyadenosine in pediatric patients with acute leukemia. J. Clin. Onc., **9**: 416 (1991).

2. Recommendations for the Safe Handling of Parenteral Antineoplastic Drugs. NIH Publication No. 83-2621. For sale by the Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 20402.

3. AMA Council Report. Guidelines for Handling Parenteral Antineoplastics, JAMA, March 15 (1985).

4. National Study Commission on Cytotoxic Exposure--Recommendations for Handling Cytotoxic Agents. Available from Louis P. Jeffrey, Sc.D., Chairman, National Study Commission on Cytotoxic Exposure, Massachusetts College of Pharmacy and Allied Health Sciences, 179 Longwood Avenue, Boston, Massachusetts 02115.

5. Clinical Oncological Society of Australia: Guidelines and Recommendations for Safe Handling of Antineoplastic Agents, Med. J. Australia **1**:425 (1983).

6. Jones RB, *et al.* Safe Handling of Chemotherapeutic Agents: A Report from the Mount Sinai Medical Center. Ca--A Cancer Journal for Clinicians, Sept/Oct. 258-263 (1983).

7. American Society of Hospital Pharmacists Technical Assistance Bulletin on Handling Cytotoxic Drugs in Hospitals. Am. J. Hosp. Pharm., **42**:131 (1985).

8. OSHA Work-Practice Guidelines for Personnel Dealing with Cytotoxic (antineoplastic) Drugs. Am. J. Hosp. Pharm., **43**:1193 (1986).

† Viaflex® containers, manufactured by Baxter Healthcare Corporation - Code No. 2B8013 (tested in 1991)

‡ MEDICATION CASSETTE™ Reservoir, manufactured by SIMS Deltec, Inc. - Reorder No. 602100A (tested in 1991)

Centocor Ortho Biotech Products, L.P. [new code]
Raritan, NJ 08869 Revised July 2012

EXHIBIT M

United States Patent and Trademark Office

Office of the Commissioner for Patents

Maintenance Fee Statement

CURRENT CORRESPONDENCE ADDRESS	CUSTOMER #	ENTITY STATUS	STATEMENT GENERATED
SALIWANCHIK, LLOYD & EISENSCHENK A PROFESSIONAL ASSOCIATION PO BOX 142950 GAINESVILLE, US 32614	23557	UNDISCOUNTED	05/19/2019 17:50:15

Invention

CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

PATENT #	APPLICATION #	FILING DATE	ISSUE DATE
7713947	11722018	06/18/2007	05/11/2010

Payment Details

PAYMENT DATE	DATE POSTED	TRANSACTION ID	ATTORNEY DOCKET #	TOTAL PAYMENT
10/16/2013	10/17/2013	101713RAMBULKS00007497504623	MERCK SERONO S.A.	$1,600.00

Fee Code	Description	Sale ID	Fee Amount
1551	MAINTENANCE FEE DUE AT 3.5 YEARS	101713RAMBULKS00007497	$1,600.00

According to the records of the United States Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed above. The payment shown above is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

EXHIBIT N

United States Patent and Trademark Office

Office of the Commissioner for Patents

Maintenance Fee Statement

CURRENT CORRESPONDENCE ADDRESS	CUSTOMER #	ENTITY STATUS	STATEMENT GENERATED
SALIWANCHIK, LLOYD & EISENSCHENK A PROFESSIONAL ASSOCIATION PO BOX 142950 GAINESVILLE, US 32614	23557	UNDISCOUNTED	05/19/2019 17:50:17

Invention

CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

PATENT #	APPLICATION #	FILING DATE	ISSUE DATE
7713947	11722018	06/18/2007	05/11/2010

Payment Details

PAYMENT DATE	DATE POSTED	TRANSACTION ID	ATTORNEY DOCKET #	TOTAL PAYMENT
10/26/2017	10/26/2017	102617INTMTFEE00008419504623		$3,600.00

Fee Code	Description	Sale ID	Fee Amount
1552	MAINTENANCE FEE DUE AT 7.5 YEARS	102617INTMTFEE00008419	$3,600.00

According to the records of the United States Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed above. The payment shown above is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

EXHIBIT O

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

IND 74,634

Serono, Inc.
Attention: Pamela Williamson Joyce, RAC
Vice President, Regulatory Affairs and Quality Assurance
One Technology Place
Rockland, MA 02370

Dear Ms. Williamson Joyce:

We acknowledge receipt of your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act. Please note the following identifying data:

IND Number Assigned:	74,634
Sponsor:	Serono, Inc.
Name of Drug:	Cladribine, oral
Date of Submission:	March 10, 2006
Date of Receipt:	March 13, 2006

Studies in humans may not be initiated until 30 days after the date of receipt shown above. If, on or before April 12, 2006, we identify deficiencies in the IND that require correction before human studies begin or that require restriction of human studies, we will notify you immediately that (1) clinical studies may not be initiated under this IND ("clinical hold") or that (2) certain restrictions apply to clinical studies under this IND ("partial clinical hold"). In the event of such notification, you must not initiate or you must restrict such studies until you have submitted information to correct the deficiencies, and we have notified you that the information you submitted is satisfactory.

It has not been our policy to object to a sponsor, upon receipt of this acknowledgement letter, either obtaining supplies of the investigational drug or shipping it to investigators listed in the IND. However, if the drug is shipped to investigators, they should be reminded that studies may not begin under the IND until 30 days after the IND receipt date or later if the IND is placed on clinical hold.

As sponsor of this IND, you are responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the implementing regulations (Title 21 of the Code of Federal Regulations). Those responsibilities include (1) reporting any unexpected fatal or life-threatening adverse experience associated with use of the drug by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]; (2) reporting any adverse experience associated with use of the drug that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]; and (3) submitting annual progress reports [21 CFR 312.33].

As required by the Food and Drug Modernization Act and the Best Pharmaceuticals for Children Act, you are also responsible for registering certain clinical trials involving your drug product in the Clinical Trials Data Bank (http://clinicaltrials.gov & http://prsinfo.clinicaltrials.gov/). If your drug is intended for the treatment of a serious or life-threatening disease or condition and you are conducting clinical trials to test its effectiveness, then you must register these trials in the Data Bank. Although not required, we encourage you to register effectiveness trials for non-serious diseases or conditions as well as non-effectiveness trials for all diseases or conditions, whether or not they are serious or life-threatening. Additional information on registering your clinical trials, including the required and optional data elements and the FDA Draft Guidance for Industry, "Information Program on Clinical Trials for Serious or Life-Threatening Diseases and Conditions," is available at the Protocol Registration System (PRS) Information Site http://prsinfo.clinicaltrials.gov/.

Please cite the IND number listed above at the top of the first page of any communications concerning this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Neurology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, call James H. Reese, Ph.D., Regulatory Project Manager, at 301-796-1136.

Sincerely,

{See appended electronic signature page}

Robbin Nighswander, R.Ph.
Supervisory Regulatory Project Manager
Division of Neurology Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Robbin Nighswander
4/5/2006 01:46:37 PM

EXHIBIT P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

NDA 22-561

NDA ACKNOWLEDGMENT

EMD Serono, Inc.
Attention: Jill P. Hillier, Ph.D.
Director, Global Regulatory Affairs
One Technology Place
Rockland, MA 02370

Dear Dr. Hillier:

We have received your new drug application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act (FDCA) for the following:

Name of Drug Product: Cladribine, oral

Date of Application: September 29, 2009

Date of Receipt: September 30, 2009

Our Reference Number: NDA 22-561

Unless we notify you within 60 days of the receipt date that the application is not sufficiently complete to permit a substantive review, we will file the application on November 30, 2009, in accordance with 21 CFR 314.101(a).

If you have not already done so, promptly submit the content of labeling [21 CFR 314.50(l)(1)(i)] in structured product labeling (SPL) format as described at http://www.fda.gov/oc/datacouncil/spl.html. Failure to submit the content of labeling in SPL format may result in a refusal-to-file action under 21 CFR 314.101(d)(3). The content of labeling must conform to the content and format requirements of revised 21 CFR 201.56-57.

The NDA number provided above should be cited at the top of the first page of all submissions to this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Neurology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

All regulatory documents submitted in paper should be three-hole punched on the left side of the page and bound. The left margin should be at least three-fourths of an inch to assure text is not obscured in the fastened area. Standard paper size (8-1/2 by 11 inches) should be used; however, it may occasionally be necessary to use individual pages larger than standard paper size. Non-standard, large pages should be folded and mounted to allow the page to be opened for review without disassembling the jacket and refolded without damage when the volume is shelved. Shipping unbound documents may result in the loss of portions of the submission or an unnecessary delay in processing which could have an adverse impact on the review of the submission. For additional information, please see http://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/DrugMasterFilesDMFs/ucm073080.htm

If you have any questions, call James H. Reese, Ph.D., RAC, Senior Regulatory Health Project Manager, at 301-796-1136.

Sincerely,

{See appended electronic signature page}

Russell Katz, MD
Director
Division of Neurology Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22561	ORIG-1	EMD SERONO INC	CLADRIBINE

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RUSSELL G KATZ
10/13/2009

EXHIBIT Q

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

NDA 022561

REGULATORY AFFAIRS

ACKNOWLEDGE REQUEST TO WITHDRAW PENDING NDA

EMD Serono, Inc.
Attention: Peter DiRoma
Vice President, Global Regulatory Affairs
One Technology Place
Rockland, MA 02370

Dear Mr. DiRoma:

We have received your August 19, 2011, correspondence on August 19, 2011, notifying us that you are withdrawing your new drug application (NDA) for cladribine tablets.

This application was filed on July 27, 2010.

In accordance with 21 CFR 314.65, this application is withdrawn as of August 19, 2011. If you decide to resubmit this application, this withdrawal will not prejudice any future decisions on filing. You may reference information contained in this withdrawn application in any resubmission.

In addition, the resubmitted application should address the deficiencies identified in our February 28, 2011, complete response letter.

If you have any questions, call LCDR Hamet Touré, Regulatory Project Manager at (301) 796-7534.

Sincerely,

{See appended electronic signature page}

Russell Katz, M. D.
Director
Division of Neurology Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

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/s/

RUSSELL G KATZ
08/22/2011

EXHIBIT R

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

NDA 22561

NDA ACKNOWLEDGMENT

EMD Serono R&D Institute, Inc.
Attention: Lynne Baron, MS, MBA
Sr. Manager, Global and Regulatory Affairs
45A Middlesex Turnpike
Billerica, MA 01821

Dear Ms. Baron:

We have received your New Drug Application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act (FDCA) for the following:

Name of Drug: Mavenclad (cladribine) 10 mg, tablet

Date of Application: May 31, 2018

Date of Receipt: May 31, 2018

Our Reference Number: NDA 22561

We note that this application has the following regulatory history:

- originally submitted on September 30, 2009,
- resubmitted May 28, 2010,
- received a Complete Response letter on February 28, 2011, and
- withdrawn on August 19, 2011.

Unless we notify you within 60 days of the receipt date that the application is not sufficiently complete to permit a substantive review, we will file the application on July 30, 2018, in accordance with 21 CFR 314.101(a).

If you have not already done so, promptly submit the content of labeling [21 CFR 314.50(l)(1)(i)] in structured product labeling (SPL) format as described at http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm. Failure to submit the content of labeling in SPL format may result in a refusal-to-file action under 21 CFR 314.101(d)(3). The content of labeling must conform to the content and format requirements of revised 21 CFR 201.56-57.

You are also responsible for complying with the applicable provisions of sections 402(i) and 402(j) of the Public Health Service Act (PHS Act) [42 USC §§ 282 (i) and (j)], which was amended by Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law No, 110-85, 121 Stat. 904).

The NDA number provided above should be cited at the top of the first page of all submissions to this application. Send all submissions to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Neurology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

Secure email is required for all email communications from FDA when confidential information (e.g., trade secrets, manufacturing, or patient information) is included in the message. To receive email communications from FDA that include confidential information (e.g., information requests, labeling revisions, courtesy copies of letters), you must establish secure email. To establish secure email with FDA, send an email request to SecureEmail@fda.hhs.gov. Please note that secure email may not be used for formal regulatory submissions to applications (except for 7-day safety reports for INDs not in eCTD format).

If you have any questions, please call me at (240) 402-2804.

Sincerely,

{See appended electronic signature page}

Sandra Folkendt
Regulatory Health Project Manager
Division of Neurology Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

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/s/

SANDRA N FOLKENDT
06/13/2018

EXHIBIT S

IND #74,634
Oral Cladribine
Serial Number Log

SER – SEQ NO	CONTENT	DATE
000	Original IND	3/10/06
001	Information Amendment	4/10/06
002	Protocol Amendment: New investigator	4/17/06
003	Type B Meeting Summary	5/4/06
004	Protocol Amendment: New Investigators	5/10/06
005	Safety Report 510002E06TUN	6/7/06
006	New Investigators Protocol Amendment	6/14/06
007	Fast Track Application	6/30/06
008	Follow Up #1 for 15 Day Alert Report of 510002E06TUN	7/5/06
009	New Investigators: Protocol Amendment	7/17/06
010	Information Amendment: Pharm / Tox	7/29/06
011	Follow Up #2 to 15 Day Alert Report of 510002E06TUN	7/26/06
012	F/U # 3 to 15 Day Alert Report of 510002E05TUN	8/3/06
013	Response to request for Information	8/9/06
014	Revised Investigator Brochure	8/9/06
015	Response to Request for Information – Pancytopenia	8/22/06
016	Response to Request for Information – Pharmacology & Toxicology	8/22/06
017	Protocol Amendment: New Investigator	8/23/06
018	Response to non-clinical request of August 10, 2006	8/24/06
019	F/U# 5 510002E06TUN	9/13/06

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SER – SEQ NO	CONTENT	DATE
020	Protocol Amendment & Response to request	9/18/06
021	Protocol Amendment: Revised 1572	9/19/06
022	15 Day Alert Report 51002E06DEU	9/21/06
023	New Protocol 26593 cross reference 5371 (SN298)	9/26/06
024	Protocol Amendment	10/12/06
025	F/U#5 to 510002E06TUN	10/25/06
026	Response to Request : Non-Clinical Report	10/27/06
027	F/U#6 to 15 Day Alert report of 510002E06TUN	11/10/06
028	F/U#7 to 15 Day Alert Report of 510002E06TUN	11/20/06
029	Information Amendment: CMC	11/21/06
030	Protocol Amendment: New Investigators	11/28/06
031	15 Day Alert Report of 510002E06TUN	12/7/06
032	Response to Request for Information	12/18/06
033	Protocol Amendment: New Investigators	12/20/06
034	Protocol Amendment: New Investigators	1/17/07
035	Initial 15 Day Alert Report for 510001E07CHE	1/24/07
036	15 Day Alert report 510001E07CAN	1/31/07
037	15 Day Alert Report 510001E07DEU (Correction see 189 in IND 45,033)	1/16/07
038	F/U 15 Day Alert Report 510001E07DEU	2/8/07
039	Response to Request for Info.	2/9/07

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040	Initial Safety Report 510001E06GBR	2/9/07
041	Initial Safety Report 510001E07ITA	2/13/07
042	Company Name Change notification	2/20/07
043	F/U#1 to 15 Day 510001E07CHE	2/16/07
044	New Investigators Cross reference BB-IND 5371	2/26/07
045	Initial 510001E07PUN	3/5/07
046	Initial 510001E06DEU	3/14/07
047	Protocol Amendment: New Investigators BB-IND 5371	3/22/07
048	Initial Report 510001E07FIN	4/9/07
049	Change in Protocol Amendment: 25643	4/23/07
050	Initial 510001E07SVK	4/27/07
051	Initial 510001E07FIN #1	4/27/07
052	F/U#2 to 510001E07DEU	5/1/07
053	Protocol Amendment: New Investigators 26593	5/4/07
054	Protocol Amendment: Revised 1572 for 25643	5/7/07
055	Initial 15 Day 510004E07CAN	5/10/07
056	F/U #1 to 15 Day 510001E07SVK	5/11/07
057	F/U#2 to 15 Day 510001E07CHE	5/16/07
058	F/U#1 to 15 Day 510001E07TUN	5/18/07
059	Protocol Amendment: New Investigator 26593	5/23/07

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060	Response to Request for Information	5/24/07
061	F/U#1 to 15 day Alert Report 510004E07CAN	5/24/07
062	Annual Report	6/11/07
063	F/U #1 to 15 Day Alert report 510001E07ITA	6/12/07
064	Initial 15 Day Alert Report 51006E07CAN	6/19/07
065	F/U#2 to 15 Day Alert Report of 510001E07SVK	6/25/07
066	Protocol Amendment 25643: Revised 1572	6/26/07
067	Protocol Amendment: New Investigator 26593	6/29/07
068	F/U#3 to 15 Day Alert Report 510001E07DEU	7/5/07
069	F/U#2 to 15 Day Alert Report 510001E07FIN	7/6/07
070	F/U#2 to 15 Day Alert Report 510001E07TUN	7/17/07
071	Initial 15 Day Alert Report 510004E07USA	7/20/07
072	Response to Request for Information	7/26/07
073	Information Amendment: CMC	7/31/07
074	F/U#2 to 510004E07CAN	8/1/07
075	Information Amendment: New Protocol 27820	8/6/07
076	F/U#1 to 510004E07USA	8/10/07
077	F/U#3 to 510001E07FIN	8/20/07
078	Protocol Amendment: New Investigator 26593	8/20/07
079	Initial 510005E07USA	9/7/07

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SER – SEQ NO	CONTENT	DATE
080	Protocol Amendment: 26593	9/25/07
081	Revised Investigator Brochure	9/27/07
082	Protocol Amendment: Revised 1572	9/28/07
083	F/U#1 to 510005E07USA	10/11/07
084	Initial 510004E07DEU	10/23/07
085	Initial 510007E07CAN	10/30/07
086	F/U#1 510004E07DEU	11/7/07
087	F/U#1 to 510007E07CAN	11/13/07
088	F/U#2 to 510004E07DEU	11/20/07
089	Initial 510010E07RUS	11/21/07
090	F/U#2 to 510007E07CAN	11/28/07
091	F/U#3 to 510004E07DEU	12/1/07
092	Cross Reference to 5371 # 26593	12/12/07
093	Response to Request for Information	12/14/07
094	Proposal for Carcinogencity Study	12/14/07
095	Information Clinical Safety Update for 26593	12/20/07
096	Initial 510003E07CHE	12/21/07
097	F/U#4 to 510004E07DEU	12/21/07
098	Protocol amendment: Revised 1572	12/21/07
099	Initial 510002E07GBR	1/3/08

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SER – SEQ NO	CONTENT	DATE
100	F/U#3 to 510001E07CHE	1/7/08
101	Information Amendment: CMC	1/25/08
102	Clinical Safety : Safety Issue Report II	1/31/08
103	Response to Request	2/7/08
104	F/U#4 to 510001E07FIN	2/20/08
105	F/U#9 510002E06TUN	2/25/08
106	F/U#1 to 510002E07GBR	3/3/08
107	Initial 510002E08GBR	3/25/08
108	F/U#1 to 510002E07CHE	3/25/08
109	Protocol Amendment: New Investigator	3/31/08
110	Protocol Amendment: New Investigator	4/18/08
111	Initial 510001E08POL	4/23/08
112	F/U#1 to 510001E08POL	5/7/08
113	Protocol Amendment # 26593	5/12/08
114	Initial 510005E08USA	6/4/08
115	Response to Request for Information	6/9/08
116	Annual Report	6/11/08
117	F/U#1 to 510005E08USA	6/12/08
118	F/U#1 to 510001E06DEU	6/24/08
119	Protocol Amendment	6/25/08

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120	Initial 510001E08TUN	7/3/08
121	F/U#1 to 510010E07RUS	7/9/08
122	F/U#2 to 510004E07USA	7/15/08
123	CMC Amendment	7/28/08
124	New Protocol 28821	7/31/08
125	New Investigators 27820	8/5/08
126	Initial 510003E08RUS	9/8/08
127	F/U#5 to 510001E07FIN	9/16/08
128	Revised Informed Consent Form 28821	9/22/08
129	New Investigator	9/24/08
130	Revised Investigators Brochure Ed. #6	9/29/08
131	F/U#1 to 510003E08RUS	9/30/08
132	Amendment #9 25643	10/8/08
133	F/U#1 to 510002E06DEU	10/14/08
134	F/U#2 & F/U#3	10/17/08
135	Amendment to CMC Amendment	10/22/08
136	Protocol Amendment: New Investigator	10/22/08
137	Initial 510012E07RUS	10/24/08
138	New Investigators 27820	11/11/08
139	F/U#1 to 510001E07CAN	11/12/08

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140	F/U#2 to 510005E08USA	11/13/08
141	General Correspondence	11/18/08
142	F/U#1 to 510001E08TUN & F/U#4 to 510004E07USA	11/18/08
143	Initial 510001E08EST	11/19/08
144	Information Amendment: SAP	11/20/08
145	Information Amendment: Trade Name Review	11/20/08
146	F/U#1 to 510006E07CAN	11/21/08
147	Initial 510004E08RUS	11/25/08
148	F/U#3 to 51000E07CAN	11/26/08
149	F/U#1 to 510001E08EST	12/5/08
150	F/U#2 to 510003E08RUS	12/8/08
151	F/U#1 to 510002E08GBR	12/9/08
152	F/U#3 to 510007E07CAN	12/10/08
153	New Investigators Protocol 26593	12/12/08
154	F/U#2 to 510001E08EST	12/16/08
155	F/U#10 510003E06TUN	12/17/08
156	F/U#5 and Initial	12/18/08
157	New Investigators 28821	12/19/08
158	F/U#11 to 510002E06TUN	12/23/08
159	F/U #1 510004E08RUS	1/2/09

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160	New Investigator 26593	1/16/09
161	New Investigator 28821	1/16/09
162	F/U#1 to 510006E08RUS	2/6/09
163	Request for Type B Pre-NDA Mtg	2/17/09
164	Request for Proprietary Name Review	2/18/09
165	CMC Amendment	2/20/09
166	New Investigators 28821	2/23/09
167	New Investigators 26593	2/23/09
168	F/U#3 to 510006E08RUS	2/23/09
169	Initial 510001E09SRB	3/3/09
170	F/U#3 to 510006E08RUS	3/17/09
171	F/U#2 to 510001E07ITA	3/18/09
172	Request for Proprietary Name review	3/18/09
173	Initial 510001E07GRC, F/U#1 to 510001E09SRB	3/19/09
174	F/U#3 to 510001E06DEU, F/U#5 to 510004E07DEU	3/25/09
175	New Inv. 28821	3/30/09
176	New Inv. 27820	3/30/09
177	Protocol Amendment #3 to ONWARD Study 26593	4/6/09
178	Pre-NDA Briefing Document	4/9/09
179	F/U#1 to 510012E07RUS	4/15/09

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180	Protocol Amendment #2 CLARITY Ext 27820	4/21/09
181	F/U#1 to 510001E07GRC	4/24/09
182	New Investigator 28821	4/24/09
183	F/U#2 to 510001E09SRB	5/5/09
184	Initial 510001E09CAN	5/12/09
185	F/U#2 to 510005E07USA	5/15/09
186	New Investigator 28821 Oracle	5/20/09
187	F/U#1 to 510001E09CAN	5/21/09
188	F/U#3 to 510005E08USA	5/26/09
189	Initial 51002E09CAN	5/27/09
190	Initial 510001E09RUS	6/1/09
191	F/U#3 to 510001E07ITA, F/U#2 to 51000E07GRC, Initial 510003E09SRB	6/4/09
192	F/U#1 to 510001E09RUS	6/8/09
193	F/U#1 to 510002E09CAN	6/9/09
194	Annual Report	6/11/09
195	CMC Amendment: 48 Months Shelf Life Ext	6/17/09
196	F/U#1 to 510003E09SRB	6/17/09
197	F/U#2 to 510003E09CAN, F/U#1 to 510001E09RUS	6/18/09
198	New Investigator 28821	6/22/09
199	F/U#3 to 510005E07USA	6/26/09

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200	Response to Request for Information	6/30/09
201	Initial 510002E09RUS	7/1/09
202	New Protocol : Clinical Safety registry	7/6/09
203	Initial 510004E09USA	7/7/09
204	Initial 510003E09USA	7/15/09
205	F/U#4 510006E08RUS	7/17/09
206	Request for Safety Mtg Risk Evaluation	7/17/09
207	F/U#2 to 510001E09CAN	7/22/09
208	Initial 510002E09ESP	7/28/09
209	Initial 510004E09RUS	7/29/09
210	New Investigators for 28821	7/31/09
211	Questions for Mtg Request	7/31/09
212	F/U#1 to 510003E09USA	8/3/09
213	Initial 510001E09ESP	8/10/09
214	F/U#1 to 510007E07CAN and Initial 510003E09CAN	8/11/09
215	F/U#1 to 510004E09RUS	8/12/09
216	F/U#1 to 510002E09RUS	8/14/09
217	New Investigators for Protocol 28821	8/18/09
218	F/U#1 to 510004E09USA	8/18/09
219	F/U#2 to 510004E09RUS	8/19/09

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SER – SEQ NO	CONTENT	DATE
220	Request for Pediatric Waiver	8/21/09
221	F/U#2 510004E09USA	8/24/09
222	F/U#3's 510001E09CAN & 510001E09SRB F/U#1 to 510002E09ESP	8/25/09
223	F/U#2 to 51000E09SRB	8/26/09
224	F/U#2 to 510004E09USA	9/1/09
225	New Investigators-Waiver Sweden/Norway	9/17/09
226	F/U#1 510001E09RUS	9/23/09
227	Response to Request for Information - 28821	10/6/09
228	F/U#2 to 510001E09ESP	10/8/09
229	F/U#5 to 510006E08RUS	10/14/09
230	Initial 510003E09FRA F/U#4 to 510004E09USA	11/4/09
231	FU 5 510004E09USA	11-12-09
232	INIT 510009E09RUS	11-18-09
233	INIT 510001E09GBR	11-23-09
234	NEW INV PROT 28821	11-30-09
235	INIT 510010E09RUS	12-9-09
236	FU 1 510001E09GBR INIT 510004E09CAN	12-16-09
237	FU 2 510006E07CAN	12-17-09
238	FU 1 510010E09RUS	12-22-09
239	FU 2 510010E09RUS	1-7-10

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240	FU 1 510003E09FRA	1-14-10
241	FU 6 510004E09USA	1-14-10
242	INIT 510001E10DEU	1-19-10
243	FU 3 510004E09RUS	1-22-10
244	INIT 510001E10USA FU 1 510004E09CAN	1-26-10
245	FU 3 510010E09RUS	1-29-10
246	FU 1 510001E10DEU INIT 510001E10RUS	2-2-10
247	FU 2 510001E09GBR	2-5-10
248	ORACLE 28821 AMEND 3	2-9-10
249	FU 1 510009E09RUS	2-9-10
250	F/U #7 510004E09USA	02/11/10
251	Investigators Brochure – Ed. 7	02/12/10
252	F/U 1 510001E10RUS	2-24-10
253	F/U 2 510001E10DEU	3-1-10
254	F/U 1 510003E09CAN	3-5-10
255	510003E10MAR INITIAL	3-12-10
256	Protocol Amendment 3 Replacement	3-16-10
257	F/U 5 510007E07CAN F/U 8 510004E09USA	3-17-10
258	F/U 2 510004E09CAN F/U 9 510004E09USA	3-22-10
259	F/U 3 5190001E10DEU Initial 510005E10USA	3-25-10

SER – SEQ NO	CONTENT	DATE
260	Initial 510006E10USA	3-26-10
261	Request for Rolling Submission	3-26-10
262	Oracle 28821 New Investigators	3-26-10
263	Initial 510012E09RUS	4-01-10
264	F/U #10 510004E09USA	4-08-10
265	F/U #2 510009E09RUS	4-09-10
266	Protocol Amendment 4 – 28821	4-20-10
267	F/U #3 510001E09GBR	4-21-10
268	F/U#2 510003E09CAN, F/U#3 510003E09SRB F/U #11 510004E09USA	4-30-10
269	Initial 7002393	5-5-10
270	F/U #5 510003E09CAN	5-12-10
271	Initial 7004670	5-14-10
272	F/U #1 510001E10USA	5-18-10
273	Initial 7005088	5-27-10
274	Initial 7006418	6-3-10
275	F/U #1 7002393	6-7-10
276	Annual Report	6-11-10
277	F/U #1 70064818	6-14-10
278	F/U #2 7006418	6-17-10
279	F/U #3 7006418	6-21-10

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280	F/U #4 7006418 F/U #1 510006E10USA	6-22-10
281	F/U #5 7006418	6-28-10
282	F/U #1 510003E10MAR	7-5-10
283	Clarity Ext. Amendment 3	7-9-10
284	F/U #2 510003E10MAR	7-15-10
285	Initial 510003E09LBN	7-19-10
286	F/U #6 7006418	7-26-10
287	F/U #7 7006418	7-30-10
288	F/U #1 510005E10USA	8-9-10
289	F/U #1 7004670 F/U #12 510004E09USA	8-12-10
290	SAP for Protocol 27820 Amendment 3	8-16-10
291	Initial 7013214 Initial 7013215	8-17-10
292	F/U #1 510003E09LBN F/U #1 7005088	8-18-10
293	F/U #2 7005088	8-19-10
294	F/U #4 510001E10DEU	8-24-10
295	F/U #13 510004E09USA	8-25-10
296	F/U #3 7005088 F/U #2 7004670	8-26-10
297	Initial 7015319	8-30-10
298	Oracle 28821 New Investigators	8-30-10
299	F/U #1 7013214 F/U #1 7013215	9-1-10

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300	Initial 7015119 & 7014989 F/U 2 510001E07CAN, F/U 4 510004E09RUS	9-2-10
301	F/U 3 510009E09RUS F/U 3 510001E07CAN	9-3-10
302	Initial 510009E07RUS, 510001E07SRB 510001E07/UKR	9-7-10
303	F/U 4 510001E09GBR, F/U 3 7004670 F/U 4 510001E07ITA, F/U 2 510006E10USA F/U 6 510006E08RUS, F/U 1 510001E07FIN	9-9-10
304	Initial 7016360, Initial 7016689	9-13-10
305	Initial 7017132	9-15-10
306	F/U #5 510001E10DEU Initial 510002E10USA	9-16-10
307	F/U 6 510001E10DEU, F/U 1 7014989 F/U 2 510005E10USA	9-17-10
308	F/U 1 7015119 F/U 4 510001E06DEU	9-20-10
309	CMC Amendment – Stats of DP	9-23-10
310	F/U 5 510001E07ITA, F/U 2 510003E09FRA F/U 3 510006E10USA, Initial 7017149, 7014718, F/U 5 7005088, F/U 6 7005088 F/U 2 510001E08POL	9-24-10
311	F/U 1 7017132	9-27-10
312	F/U 2 7002393, F/U 2 7005319 F/U 3 7015319, F/U 2 7017132	9-30-10
313	F/U 1 7016360	10-5-10
314	F/U 6 7005088	10-6-10
315	F/U 5 510001E09GBR	10-8-10
316	F/U 4 7015319	10-15-10
317	F/U 2 510002E09RUS, Initial 7021039 F/U 1 7014718, F/U 3 7017132	10-19-10
318	F/U 1 7017149	10-22-10

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319	Initial 7019448	10-25-10
320	F/U 4 7017132	10-26-10
321	F/U 1 7021039	10-28-10
322	F/U 7 510001E09GBR	11-1-10
323	F/U 8 7006418, F/U 2 7016360 Initial 7023305, F/U 2 7021039	11-2-10
324	F/U 3 7019448	11-3-10
325	F/U 9 7006418	11-5-10
326	F/U 2 7013214, F/U 2 7013215	11-11-10
327	F/U 5 7017132 F/U 2 7017149	11-17-10
328	F/U 10 7006418	11-18-10
329	F/U 3 7021039	11-19-10
330	F/U 6 510001E07ITA, F/U 1 7023305, Initial 7026942	11-22-10
331	F/U 4 510009E09RUS F/U 1 7006418	11-23-10
332	F/U 5 510009E09RUS F/U 5 7015319	11-24-10
333	F/U 4 7004670	12-1-10
334	F/U 2 7014718 Initial 7028748	12-2-10
335	F/U 5 7004670	12-8-10
336	F/U 1 7028748	12-9-10
337	F/U 3 510003E09FRA	12-10-10
338	F/U 7 510006E08RUS	12-13-10

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339	F/U 3 7014718, F/U 1 7030335 Initial 7030335	12-14-10
340	F/U 6 7017132	12-21-10
341	F/U 3 51000E09CAN, F/U 12 7006418 F/U 6 7015319	12-23-10
342	F/U 4 510001E07CHE F/U 6 510009E09Rus	12-29-10
343	F/U 2 7030335	1-5-11
344	Revised IB v8.0	1-14-11
345	F/U 4 7021039	1-19-11
346	F/U 5 7021039	1-25-11
347	F/U 4 510005E07USA	1-28-11
348	Initial 7040290 F/U 6 7021039	2-12-11
349	F/U 7 7021039 Initial 7041399	2-23-11
350	General Corresp. Oracle CTFG	2-28-11
351	New Investigators: Oracle 28821 & Onward 26593	3-2-11
352	F/U 1 7041399 Initial 7043041	3-3-11
353	F/U 1 7043041, F/U 2 7043041 F/U 3 7016360	3-4-11
354	F/U 2 7041399	3-9-11
355	F/U 3 7043041 F/U 2 510002E10USA	3-14-11
356	Initial 7039801 Initial 7047529	3-22-11
357	F/U 7 7015319, F/U 2 7023305 F/U 1 7047529	3-28-11
358	Initial 7047972 F/U 8 7015319	3-29-11

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359	F/U 1 7039801	3-30-11
360	Revised ICF: 26593, 28821 & Premiere	3-31-11
361	F/U 3 7023305	4-4-11
362	F/U 4 5100010e09rus	4-6-11
363	New Non-IND Investigators: 28821	4-6-11
364	F/U 13 7006418 Initial 7044421	4-7-11
365	F/U 5 510003E10MAR	4-12-11
366	F/U 2 7039801 F/U 2 7047529	4-13-11
367	Clarity Ext 27820, Amendment 4 & Revised ICF	4-13-11
368	F/U 4 510003E10MAR, F/U 3 & 4 7047529	4-15-11
369	F/U 1 7044421	4-21-11
370	F/U 14 7006418	4-25-11
371	F/U 5 510003E10MAR F/U 2 7014989	4-26-11
372	F/U 5 7047529	4-27-11
373	F/U 6 7047529 Initial 7052709	5-4-11
374	F/U 4 7016360	5-10-11
375	Initial 7057609 Initial 7057926	5-16-11
376	F/U 1 7047972 Initial 7059309	5-24-11
377	Initial 7053426, Initial 7060061 Initial 7060149	5-27-11
378	F/U 8 7021039, F/U 1 7059309 F/U 7 7005088, F/U 7 7047529, F/U 1 7057926	6-1-11

SER – SEQ NO	CONTENT	DATE
379	F/U 3 7013214 F/U 3 7013215	6-2-11
380	Initial 7061169 Initial 7061816	6-6-11
381	F/U 1 7060149 F/U 1 7053426	6-8-11
382	Annual Report	6-9-11
383	F/U 1 7057609	6-13-11
384	F/U 8 7047529 Initial 7063170	6-16-11
385	F/U 2 7057609 Initial 7063793	6-17-11
386	Initial 7064728	6-22-11
387	F/U 2 7060149 F/U 1 7063170	6-28-11
388	Initial 7065584	6-30-11
389	F/U 1 7052709 F/U 1 7063793	7-1-11
390	Initial 7068227	7-13-11
391	F/U 4 510004E09CAN, F/U 3 7014989 F/U 2 7059309	7-20-11
392	F/U 5 5100109RUS F/U 2 7047972	7-25-11
393	F/U 14 510004E09USA, F/U 9 7015319 F/U 3 7059309	8-1-11
394	F/U 1 7065584 F/U 2 7052709	8/3/11
395	Initial 7072634	8/8/11
396	F/U 3 7047972	8-10-11
397	Initial 7073386 F/U 2 7065584	8-11-11
398	Initial 7045053 F/U 1 7072634	8-16-11

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SER – SEQ NO	CONTENT	DATE
399	F/U 15 510004E09USA F/U 6 510010E09RUS	8-22-11
400	F/U 4 7059309	8-31-11
401	F/U 6 510003E10MAR	9-6-11
402	F/U 5 7059309, Initial 7060478 & 7076477, F/U 1 7073386	9-14-11
403	Amendment 5 for 28821, 26593 & rev ICF's for Premiere Registry	9-16-11
404	Initial 7069384 & 7076391	9-19-11
405	F/U 4 7047972 F/U 6 7059309	9-22-11
406	F/U 1 7064228	10-13-11
407	F/U 4 7023305, F/U 3 7041399, F/U 7 7059309, F/U 8 7059309 F/U 3 7065584, F/U 4 7065584, F/U 2 7073386	10-18-11
408	F/U 3 7039801, F/U 4 7041399, Initials 7062063, 7092606, F/U 2 7063793	11-8-1
409	Clin Info Amendment terminating Onward & Oracle Studies	11-10-11
410	Initial 7093624	11-15-11
411	Initial 7094333	11-21-11
412	F/U 4 7014718, F/U 9 7059309	12-5-11
413	F/U 9 7005088, F/U 5 7014718, F/U 1 7093624, F/U 1 7094333	12-7-11
414	F/U 6 7014718, F/U 7 7014718, F/U 3 7072634, F/U 2 7072634	12-21-11
415	F/U 10 7059309	12-27-11
416	F/U 2 7094333, F/U 1 7101799, F/U 2 7094333	12-30-11
417	F/U 5 7047972, Initial 7101970	1-5-12
418	F/U 1 7040290, F/U 4 7072634	1-18-12

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Oral Cladribine
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SER – SEQ NO	CONTENT	DATE
419	Initial 7108211	1-27-12
420	Initials 510002e09POL & 7108209, F/U 1 7101970, F/U 2 7045053	1-30-12
421	F/U 1 7108209, F/U 1 7108211	1-31-12
422	F/U 2 7101799	2-10-12
423	F/U 2 7101970, F/U 2 7108209	2-16-12
424	F/U 3 7101970	2-23-12
425	F/U 3 7060149, Initials 7113253 & 7113255, F/U 1 7113253	2-27-12
426	Premiere Protocol Amendment & revised ICF's	3-2-12
427	Initial 7115887	3-12-12
428	Initial 7047529	3-27-12
429	Premiere Amendment 1 Tracked Version	4-4-12
430	Initial 7121036, F/U 1 7121036	4-4-12
431	2 aCSRs – Oracle & Clarity Ext	4-6-12
432	F/U 5 510005E07USA	4-10-12
433	F/U 2 7057926	4-11-12
434	F/U 2 7093624	4-13-12
435	F/U 5 7023305 Initial 7125405	4-24-12
436	F/U 16 510004E09USA	4-26-12
437	F/U 17 510004E09USA F/U 3 7045053	5-1-12
438	F/U 11 7059309	5-23-12

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Oral Cladribine
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SER – SEQ NO	CONTENT	DATE
439	Initial 7133572	5-30-12
440	Initial 7133768 F/U 5 7016360	6-1-12
441	Initials 7134689, 7134687, 7135032	6-6-12
442	Initial 7135880, F/U 4 7043041 F/U 3 7094333	6-7-12
443	Annual Report	6-8-12
444	Initial 7138299	6-12-12
445	F/U 1 7138299 F/U 3 7017149	6-13-12
446	F/U 1 7016689	6-21-12
447	Initial 7134374	6-22-12
448	Initial 7142690	7-5-12
449	F/U 1 7142690 Initial 7145323	7-11-12
450	Initial 7144397 F/U 1 7144397 Movectro	7-12-12
451	Premiere Amendment 1, V3 & Rev. ICFs	7-30-12
452	Initial 7149516	8-8-12
453	Initial 7152077	8-13-12
454	F/U 1 7149516	8-14-12
455	Initial 7154094	8-23-12
456	F/U 4 7045053	9-6-12
457	F/U 2 7019448	9-26-12
458	Clarity Extension 120 Week Report	10-1-12

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SER – SEQ NO	CONTENT	DATE
459	F/U 3 7019448	10-4-12
460	F/U 1 7076477, F/U 5 510004e09can, Initial 7166798 F/U 1 7154094, F/U 1 7166798	10-23-12
461	Initial 7172246	11-9-12
462	F/U 2 7154094	11-21-12
463	F/U 1 7172246	12-3-12
464	F/U 7 7017132 F/U 6 7047972	12-11-12
465	Cross Ref for Cladribine Ctrs 28821 & 26593	1-14-13
466	Initials 7187173 & 7187166	1-24-13
467	F/U 1 7187173	2-4-13
468	F/U 3 7154094	2-21-13
469	Initial 7197251	3-6-13
470	F/U 1 7197251, Initials 7197832 & 7197826	3-12-13
471	Initial 7197867, F/U 1 7197867	3-20-13
472	F/U 1 7187166, F/U 4 7094333	3-27-13
473	F/U 2 7197251	4-2-13
474	F/U 7152077	4-12-13
475	F/U 3 7197251	4-16-13
476	F/U 2 7187173, F/U 2 7197867	4-30-13
477	Initial 7207635	5-2-13
478	Initial 7211838	5-29-13

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SER – SEQ NO	CONTENT	DATE
479 - 436	Anual Report	6-5-13
480 – 437	Initial 7215739, F/U 1 7215739 F/U 1 7211838	6-11-13
481 - 438	F/U 2 7211838	6-25-13
482 – 439	Initial 7221576 F/U 1 7221576	7-11-13
483 – 440	F/U 2 7221576	8-2-13
484 - 441	F/U 4 510006E10USA	9-4-13
485 – 442	F/U 1 7207635	9-12-13
486 – 443	F/U 2 7207635	9-25-13
487 – 444	F/U 3 7221576	10-7-13
488 - 445	F/U 2 7215739	10-16-13
489 – 446	F/U 3 7152077 F/U 4 7152077	12-3-13
490 – 447	F/U 3 7197867	2-14-14
491 – 448	F/U 4 7197867	3-7-14
492 – 449	Annual Report	6-9-14
493 – 450	Initial 7310422	8-12-14
494 – 451	Initial 7310877 postmarketing australia	8-13-14
495 – 452	F/U 1 7310422	8-19-14
496 – 453	Initial 7315281	9-2-14
497 – 454	Initial 8006474	2-3-15
498 – 455	8006474-1	2-6-15

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499 – 456	7142690-2	3-11-15
500 – 457	7315281-1	3-17-15
501 – 458	8016716-0	3-31-15
502 – 459	7187173-3 7101970-4	5-14-15
503 – 460	Annual Report (2014-2015)	6-11-15
504 – 461	7315281-2	6-19-15
505 – 462	8016716-1	8-6-15
506 – 463	7187173-43	9-11-15
507 – 464	7154094-4	9-17-15
508 – 465	8045741-0	10-6-15
509 – 466	7108209-3	2-4-16
510 – 467	7197251-4 7144397-2	3-4-16
511 - 468	7142690-3 7315281-3	3-16-16
512 – 469	7207635-3	3-23-16
513 – 470	510009e09rus-7	3-30-16
514 – 471	7197832-1	4-1-16
515 – 472	510010e09rus-7 (late-was due 4-2; capa was sent)	4-4-16
516 - 473	7211838-3	4-7-16
517 – 474	8078649-0 8006474-2 71871735 7207635-4	4-13-16
518 – 475	7144397-3	4-20-16

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519 – 476	7187173-6 015	5-11-16
520 – 477	8078649-1 012	5-13-16
521 – 478	Request for DSUR	6-6-16
522 – 479	7310877-1 postmarketing australia	7-13-16
523 – 480	DSUR 2015-2016	8-31-16
524 – 481	7310877-2 postmarketing australia	9-13-16
525-482	Protocol amendment	9-22
526-483	Request for revised DSUR due date	10-14
527-484	8045741-1 (012)	10-25
528-485	7207635-5 7154094-5 (012)	12-27
529-486	8147577-0 (27820)	3-17-17
530-487	8147577-1 (27820)	3-24-17
531-488	8147577-2 (27820)	4-18-17
532-489	Type C meeting requenst	5-15
533-490	8162959-0 (27820)	6-16
534-491	Change in contact – Lynne Baron	6-30
535-492	8045741-2 (012)	7-19
536-493	Request for proprietary name review	7-21
537-494	8162959-1	8-18
538-495	DSUR 2016-2017	9-1

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539-496	Info Amend: Clinical - IB update 1.0	9-21
540-497	PSP – pediatric study plan	11-1
541-498	8199968-0	11-17
542-499	8203690-0	11-22
543-500	8203487-0	11-29
544-501	8205831-0	11-30
545-502	8203690-1	12-15
546-503	**90003603-0**	**1-2-18**
547-504	9001728-0 9008213-0	1-31
548-505	9008718-0	2-6
549-506	9010286-0	2-15
550-507	9008718-1	2-23
551-508	9010286-1	2-26
552-509	9008718-2 9012465-0	2-28
553-510	9008718-3	3-12
554-511	General corres	3-20
555-512	9008213-1	3-20
556-513	9016723-0 9008718-4 9009984-0	3-23
557-514	9008213-2	3-28
558-515	Type B mtg request PPMS	4-4

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559-516	9016723-1	4-6
560-517	9016723-2	4-19
561-518	Briefing Book	4-23
562-519	Pediatric Study Plan - PSP	4-24
563-520	9023286-0	5-10
564-521	Revised PSP	5-14
565-522	9008213-3 9023286-1	5-16
566-523	9026775-0 90038366-0	5-18
567-524	90038366-1 90038366-2	6-1
568-525	9029591-0 90038366-3 9029837-0 9029831-0 9030166-0	6-12
569-526	9031080-0 9032015-0 9032873-0	6-29
570-527	9032987-0 90050647-0 9031080-1 9035563-0	7-17
571-528	9035496-0	7-25
572-529	Update module 3 & 4; chg in contact from Lynn B to Tammy S	7-31
573-530	9035563-1 9037212-0 9034720-0	8-1
574-531	9037212-1	8-8
575-532	9035636-0 9039790-0	8-21
576-533	9039995-0 9040223-0	8-28
577-534	009-9035636-1 9040223-1 PSP 90054939-0	8-30
578-535	DSUR 2017-2018	9-5

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579-536	9036764-0 9040223-2 9041724-0 9041833-0 90024448-0	9-7
580-537	9042393-0 9042178-0 9042546-0 90054939-1	9-14
581-538	9043767-0 9044115-0 9029837-1 9044324-0	9-25
582-539	9043919-0 9035636-2 9044992-0 9042546-1	10-3
583-540	9044324-1 9045939-0 9045721-0 9045719-0 9039790-1 9036764-1 9045753-0 9035636-3 9017898-0	10-9
584-541	9046711-0 9039931-0 90055540-0	10-11
585-542	9040223-3	10-12
586-543	9039995-1	10-17
587-544	9046995-0 9046966-0 9046675-0 9029837-2	10-19
588-545	9043767-1 9047436-0 9046711-1	10-23
589-546	9036764-2 9046571-0 9044449-0 9035636-4	10-31
590-547	8006474-0 9043767-2 9046995-1 9049467-0 9043762-2	11-5
591-548	9045376-0 9000101-1 9008718-5	11-7
592-549	Amendment non clinical	11-9
593-550	90356365 9050599-0 9050228-0 9032820-0	11-14
594-551	9045865-0 9044992-1 9043557-0	11-20
595-552	9052502-0 9012465-1 9046675-1 9045376-1 9046711-2 9035636-6	11-27
596-553	9009984-1 9029831-1 9054946-0 9050228-1	11-30
597-554	9042393-1 9032820-2	12-6
598-555	9052502-1 9057542-0 9046571-1 9046711-3 90065122-0	12-18

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599-556	9058502-0 9059148-0 9057843-0 90065359-0	12-21-18
600-557	9053378-0 9056311-0 9055198-0	12-28-18
601-558	PSP request for feedback	**1-7-19**
602-559	9062762-0 9062762-1 9062255-0 9061546-0 9057542-1 9063072-0 9063208-0 9032987-1	1-17-19
603-560	9065109-0 9032987-1 9064142-0 9064183-0 9065064-0 9065022-0 9060359-0 9053378-1	1-24-19
604-561	9063208-1 9062762-2 9065795-0 90020189-0 9056500-0 9035659-0	1-29-19
605-562	9042546-2 90020189-1 9067688-0 9035636-7	2-5
606-563	9069368-0 9035636-8 9070247-0 9009984-2 9062255-1 90054939-2 9061546-1	2-15
607-564	9054192-6 9055198-1 9061546-2 9041193-0 9050228-2 9017898-1 9072028-0 9072025-0	2-22
608-565	9072945-0 9062104-0 9073084-0 9063358-0 9069368-1 9070247-1 9056311-1 9044115-1	3-1
609-566	9074360-0 9040223-4 9075013-0 9074964-0 9017898-2 9050228-3 9072025-1	3-8
610-567	9075013-1 9062104-1	3-15
611-568	9070552-0 9061546-3 9041193-1	3-22
612-569	90062992-0 9061546-4 9079022-0 9065637-1 9079698-0 9072028-1	3-27
613-570	CMC Amendment	4-1
614-571	9078568-1 9080621-0 90038366-4	4-5
615-572	9054753-0 9042178-1	4-12
616-573	9081819-0 9028817-0 90062992-1	4-17
617-574	9061546-5 - 0021	4-19

NDA 022561US Chronology

Date	Content
September 29, 2009	Application Dated
September 30, 2009	Application Received
October 13, 2009	FDA Acknowledgment of NDA receipt signed
November 19, 2009	Tradename submission
November 25, 2009	FDA Refuse to file Received
February 22, 2010	End-of-Review Meeting summary
March 25, 2010	Teleconference to discuss structure and content for NDA resubmission
April 27, 2010	Rolling submission Part 1/2
May 27, 2010	Rolling submission Part 2/2
May 28, 2010	Tradename resubmission
May 28, 2010	Response to FDA Information request
June 24, 2010	Response to FDA Information request
June 28, 2010	Response to FDA Information request
July 2, 2010	Response to FDA Information request
July 20, 2010	Response to FDA Information request
July 21, 2010	Response to FDA Information request (2)
July 30, 2010	Response to FDA Information request
August 16, 2010	Response to FDA Information request
August 18, 2010	Response to FDA Information request
September 1, 2010	Response to FDA Information request
September 2, 2010	Response to FDA Information request
September 8, 2010	Response to FDA Information request (2)
September 10, 2010	Response to FDA Information request
September 13, 2010	Response to FDA Information request
September 15, 2010	Response to FDA Information request
September 17, 2010	Response to FDA Information request (2)
September 22, 2010	Response to FDA Information request
September 23, 2010	Response to FDA Information request
September 29, 2010	Response to FDA Information request

Date	Content
September 30, 2010	Response to FDA Information request (2)
October 1, 2010	Response to FDA Information request
October 4, 2010	Response to FDA Information request
October 12, 2010	Response to FDA Information request
October 13, 2010	Response to FDA Information request (3)
October 18, 2010	Response to FDA Information request
October 28, 2010	Response to FDA Information request
November 1, 2010	Response to FDA Information request
November 5, 2010	Response to FDA Information request
November 9, 2010	Response to FDA Information request
November 10, 2010	Response to FDA Information request
December 1, 2010	Response to FDA Information request
February 28, 2011	Complete Response from FDA
March 3, 2011	Complete Response acknowledgment
June 8, 2011	End-of-Review Meeting summary
August 19, 2011	Application Withdrawn
August 22, 2011	FDA Acknowledgment of withdrawal signed
August 25, 2011	FDA Acknowledgment of withdrawal received
May 15, 2017	Type C meeting request submitted
May 26, 2017	Type C meeting request rejected
May 30, 2017	Teleconference to discuss resubmission
October 12, 2017	FDA Pre-submission Meeting
May 30, 2018	Application Dated
May 31, 2018	Application Received
June 3, 2018	FDA Acknowledgment of NDA receipt (including acknowledgment of response to all items listed in February 28, 2011, Complete Response letter) signed
August 10, 2018	Response to FDA Information request
August 28, 2018	Response to FDA Information request
November 6, 2018	Response to FDA Information request
November 9, 2018	Response to FDA Information request

Date	Content
December 5, 2018	Response to FDA Information request
December 12, 2018	Response to FDA Information request
December 19, 2018	Response to FDA Information request
January 10, 2019	Response to FDA Information request
January 25, 2019	Response to FDA Information request
January 30, 2019	Carton and container labeling submitted to FDA
February 8, 2019	Response to FDA Information request
February 19, 2019	Response to FDA Information request
March 1, 2019	Response to FDA Information request
March 5, 2019	Response to FDA Information request
March 14, 2019	Draft Label received
March 18, 2019	Response to FDA Information request
March 19, 2019	Timetable for study submitted to FDA
March 21, 2019	Timetable for study submitted to FDA
March 22, 2019	Draft Label received
March 25, 2019	Teleconference with FDA to discuss label
March 27, 2019	Draft Label received
March 28, 2019	Response to FDA Information request
March 29, 2019	NDA Approval signed

United States Patent and Trademark Office

- Sales Receipt -

06/07/2019 GARIAS 00000001 601920 11722018

01 FC:1457 1120.00 DA

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22314-1450
www.uspto.gov

Food and Drug Administration
CDER, Office of Regulatory Policy
10903 New Hampshire Avenue,
Bldg. 51 Room 6250
Silver Spring MD 20993-0002

September 4, 2020

Attention: Beverly Friedman

The attached application for patent term extension of U.S. Patent No. 7,713,947 was filed on May 24, 2019, under 35 U.S.C. § 156.

The assistance of your Office is requested in confirming that the product identified in the application Mavenclad® (cladribine), has been subject to a regulatory review period within the meaning of 35 U.S.C. § 156(g) before its first commercial marketing or use and that the application for patent term extension was filed within the sixty-day period beginning on the date the product was approved. Since a determination has not been made whether the patent in question claims a product which has been subject to the Federal Food, Drug and Cosmetic Act, or a method of manufacturing or use of such a product, this communication is NOT to be considered as notice which may be made in the future pursuant to 35 U.S.C. § 156(d)(2)(A).

Our review of the application to date indicates that the subject patent would be eligible for extension of the patent term under 35 U.S.C. § 156.

Inquiries regarding this communication should be directed to the undersigned at (571) 272-0909 (telephone) or (571) 273-0909 (facsimile) or by e-mail at ali.salimi@uspto.gov.

/Ali Salimi/

Ali Salimi
Senior Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Dr. Kirsten Grüneberg
Grüneberg and Myers, PLLC
1775 Tysons Blvd
5th Floor
Tysons, VA 22102

U.S. FOOD & DRUG ADMINISTRATION

Re: MAVENCLAD
Patent No. 7,713,947
Docket No. FDA-2020-E-1885

The Honorable Andrei Iancu
Under Secretary of Commerce for Intellectual Property
Director, United States Patent and Trademark Office
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

OCT 13 2020

Dear Director Iancu:

This is concerning the application for patent term extension for U.S. Patent No. 7,713,947 filed by Merck Serono SA, under 35 U.S.C. 156. The human drug product claimed by the patent is MAVENCLAD (cladribine), which was assigned new drug application (NDA) No. 22561.

A review of the Food and Drug Administration's official records indicates that this product was subject to a regulatory review period before its commercial marketing or use, as required under 35 U.S.C. 156(a)(4). However, our records also indicate that MAVENCLAD (cladribine) does not represent the first permitted commercial marketing or use of the product, as defined under 35 U.S.C. § 156(f)(1).

The active ingredient in MAVENCLAD, cladribine, has been previously approved for commercial marketing or use, in the following list[1] of prior NDA and abbreviated NDA (ANDA) approvals:

PRODUCT NAME (GENERIC NAME)	APPLICATION NUMBER	APPLICANT	APPROVAL DATE
LEUSTATIN (CLADRIBINE)	NDA 20229	Janssen Pharmaceuticals Inc.	2/26/1993
CLADRIBINE	ANDA 75405	West-Ward Pharmaceuticals International Ltd.	2/28/2000
CLADRIBINE	ANDA 76571	Fresenius Kabi USA LLC	4/22/2004
CLADRIBINE	ANDA 200510	Mylan Laboratories Ltd.	10/6/2011

[1] Not comprehensive.

U.S. Food and Drug Administration
10903 New Hampshire Avenue
WO Building 51, Room 6250
Silver Spring, MD 20993-0002
www.fda.gov

NDA 22561 was approved on March 29, 2019, which makes the submission of the patent term extension application on May 24, 2019, timely within the meaning of 35 U.S.C. 156(d)(1).

Should you conclude that the subject patent is eligible for patent term extension, please advise us accordingly. As required by 35 U.S.C. 156(d)(2)(A) we will then determine the applicable regulatory review period, publish the determination in the *Federal Register*, and notify you of our determination.

Please let me know if we can be of further assistance.

Sincerely yours,

Patrizia Cavazzoni

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In Re: Patent Term Extension
Application for
U.S. Patent No. 7,713,947

May 20, 2021

NOTICE OF DETERMINATION OF INELIGIBILITY

An application for extension of the patent term of U.S. Patent No. 7,713,947 (the '947 patent) under 35 U.S.C. § 156 was filed in the United States Patent and Trademark Office on May 24, 2019. The application was filed by Merck Serono SA. Extension is sought based upon the premarket review under § 505 of the Federal Food, Drug, and Cosmetic Act (FFDCA) of New Drug Application (NDA) 22561 for the human drug product known by the tradename Mavenclad® (cladribine) having the active ingredient cladribine. Mavenclad® (cladribine) was approved for commercial use and sale by the Food and Drug Administration (FDA) on April 1, 2019.

A determination has been made that U.S. Patent No. 7,713,947 is **NOT** eligible for patent term extension under 35 U.S.C. § 156 based upon the regulatory review period of Mavenclad® (cladribine) which is the subject of NDA 22561.

A single request for reconsideration of this FINAL DETERMINATION OF INELIGIBILITY may be made if filed by the applicant within TWO MONTHS of the mailing date of this letter. The period for response may be extended pursuant to 37 C.F.R. 1.136. See 37 C.F.R. 1.750. A failure to respond to this letter will result in the application papers being placed into the patent file with no further action taken on the application for patent term extension.

I. The Approval of Mavenclad® As Claimed In The '947 patent Fails to Comply with 35 U.S.C. § 156(a)(5)(A)

The FDA official records indicate that cladribine was previously approved for commercial marketing or use prior to the approval of Mavenclad® (cladribine). In a letter dated October 13, 2020, FDA stated:

> A review of the Food and Drug Administration's official records indicates that this product was subject to a regulatory review period before its commercial marketing or use, as required under 35 U.S.C. § 156(a)(4). However, our records also indicate that Mavenclad (cladribine) **does not** represent the first permitted commercial marketing or use of the product, as defined under 35 U.S.C. § 156(f)(1). The active ingredient in

Mavenclad, cladribine,[1] has been previously approved in the following list[2] of prior NDA, and abbreviated NDA (ANDA) approvals:

PRODUCT NAME (GENERIC NAME)	APPLICATION NUMBER	APPLICANT	APPROVAL DATE
LEUSTATIN (CLADRIBINE)	NDA 20229	Janssen Pharmaceuticals Inc.	2/26/1993
CLADRIBINE	ANDA 75405	West-Ward Pharmaceuticals International Ltd.	2/28/2000
CLADRIBINE	ANDA 76571	Fresenius Kabi USA LLC	4/22/2004
CLADRIBINE	ANDA 200510	Mylan Laboratories Ltd.	10/6/2011

Under 35 U.S.C. § 156(a) a term of a patent which claims a product shall be extended if, *inter alia*, the product has been subject to a regulatory review period before its commercial marketing or use. In addition, under § 156(a)(5)(A):

> the permission for the commercial marketing or use of the product . . . is the first permitted commercial marketing or use of the product under the provision of law under which such regulatory review period occurred; (Emphasis added)

Thus, the determination of eligibility of U.S. Patent No. 7,713,947 turns on the provisions in § 156(a)(5)(A) that the permission for the commercial marketing or use is **the first** permitted commercial marketing or use of the product. The term "product" is defined in 35 U.S.C. § 156(f) as follows:

> (f) For purposes of this section:
> (1) The term "product" means:
> (A) A drug product . . .
> (2) The term "drug product" means the active ingredient of -
> (A) A new drug, antibiotic drug, or human biological product . . . .including any salt or ester of the active ingredient, as a single entity or in combination with another active ingredient. (Emphasis added.)

By the explicit terms of section 156(f)(2), the term "product" as it relates to a human drug product means the active ingredient of the new drug product. The active ingredient in the approved product, is cladribine. As noted in the above FDA letter, the active ingredient

[1] As per the October 13, 2020 letter, FDA has confirmed that the active ingredient in the product is cladribine alone.

[2] Not comprehensive

cladribine had been approved for commercial marketing and use prior to the approval of the applicant's product. Furthermore, the prior approval of the active ingredient cladribine by the Food and Drug Administration was under section 505 of the FFDCA, the same provision of law under which regulatory review of NDA 22561 for Mavenclad® (cladribine) occurred. Applying the definition of "product" provided in section 156(f) to the extension requirement of § 156(a)(5)(A), applicant's product, Mavenclad® (cladribine), does not qualify as the first permitted marketing or use of the active ingredient. Since the approval of Mavenclad® (cladribine) was not the first permitted marketing or use of the active ingredient thereof, cladribine, the patent is not eligible for patent term extension based upon the regulatory review of Mavenclad® (cladribine). See *In re Fisons Pharmaceuticals Limited*, 231 USPQ 305 (Comm'r Pats. 1986); aff'd, *Fisons plc v. Quigg*, 8 USPQ2d 1491 (DDC 1988); aff'd, 10 USPQ2d 1869 (Fed. Cir. 1988); *Glaxo Operations UK Ltd. v. Quigg*, 13 USPQ 1628 (Fed. Cir. 1990).

II. Conclusion

For the above-stated reason, the PTE application for the 7,713,947 patent is **DISMISSED**.

Any correspondence from applicant with respect to this matter should be submitted via the USPTO's EFS Web system and should be addressed as follows:

By mail: Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450.

Telephone inquiries related to this determination should be directed to the undersigned at (571) 272-0909.

/Ali Salimi/

Ali Salimi
Senior Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: FDA, CDER, Office of Regulatory Policy
10903 New Hampshire Avenue,
Bldg. 51 Room 6250
Silver Spring MD 20993-0002

Attention: Beverly Friedman

RE: Mavenclad® (cladribine)
Docket No.: FDA-2020-E-1885

EXHIBIT H

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use MAVENCLAD safely and effectively. See full prescribing information for MAVENCLAD.

MAVENCLAD® (cladribine) tablets, for oral use
Initial U.S. Approval: 1993

WARNING: MALIGNANCIES and RISK OF TERATOGENICITY
See full prescribing information for complete boxed warning.

- **Malignancies**

MAVENCLAD may increase the risk of malignancy. MAVENCLAD is contraindicated in patients with current malignancy; evaluate the benefits and risks on an individual basis for patients with prior or increased risk of malignancy. (5.1)

- **Risk of Teratogenicity**

MAVENCLAD is contraindicated for use in pregnant women and in women and men of reproductive potential who do not plan to use effective contraception because of the risk of fetal harm. (5.2)

----------INDICATIONS AND USAGE----------

MAVENCLAD is a purine antimetabolite indicated for the treatment of relapsing forms of multiple sclerosis (MS), to include relapsing-remitting disease and active secondary progressive disease, in adults. Because of its safety profile, use of MAVENCLAD is generally recommended for patients who have had an inadequate response to, or are unable to tolerate, an alternate drug indicated for the treatment of MS *[see Warnings and Precautions (5)]*. (1)

Limitations of Use

MAVENCLAD is not recommended for use in patients with clinically isolated syndrome (CIS) because of its safety profile *[see Warnings and Precautions (5)]*. (1)

----------DOSAGE AND ADMINISTRATION----------

- Assessments are required prior to starting each MAVENCLAD treatment course. (2.1)
- Cumulative dosage of 3.5 mg/kg administered orally and divided into 2 treatment courses (1.75 mg/kg per treatment course). Each treatment course is divided into 2 treatment cycles. (2.2)
- MAVENCLAD is a cytotoxic drug. (2.4)
- Separate administration from any other oral drug by at least 3 hours. (2.4)

----------DOSAGE FORMS AND STRENGTHS----------

Tablets: 10 mg (3)

----------CONTRAINDICATIONS----------

- Patients with current malignancy. (4)
- Pregnant women, and women and men of reproductive potential who do not plan to use effective contraception during MAVENCLAD dosing and for 6 months after the last dose in each treatment course. (4, 8.3)
- HIV infection. (4)
- Active chronic infections (e.g., hepatitis or tuberculosis). (4)
- History of hypersensitivity to cladribine. (4, 5.8)
- Women intending to breastfeed on a MAVENCLAD treatment day and for 10 days after the last dose. (4, 8.2)

----------WARNINGS AND PRECAUTIONS----------

- Lymphopenia: Monitor lymphocyte counts before, during and after treatment. (5.3)
- Infections: Screen patients for latent infections; consider delaying treatment until infection is fully controlled. Vaccinate patients antibody-negative to varicella zoster virus prior to treatment. Administer anti-herpes prophylaxis in patients with lymphocyte counts less than 200 cells per microliter. Monitor for infections. (5.4)
- Hematologic toxicity: Measure complete blood count annually if clinically indicated after treatment. (5.5)
- Graft-versus-host-disease with blood transfusion: Irradiation of cellular blood components is recommended. (5.6)
- Liver injury: Obtain tests prior to treatment. Discontinue if clinically significant injury is suspected. (5.7)

----------ADVERSE REACTIONS----------

Most common adverse reactions (incidence > 20%) are upper respiratory tract infection, headache, and lymphopenia. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact EMD Serono at 1-800-283-8088 ext. 5563 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------DRUG INTERACTIONS----------

- Immunosuppressive drugs: Consider overlapping effects on immune system, when used sequentially. Concomitant use not recommended. (7.1)
- Hematotoxic drugs: Monitor patients for additive effects on the hematological profile. (7.3)
- Antiviral and antiretroviral drugs: Avoid concomitant use. (7.4)
- BCRP or ENT/CNT inhibitors: May alter bioavailability of cladribine. Avoid concomitant use. (7.5)

See 17 for PATIENT COUNSELING INFORMATION **and Medication Guide.**

Revised: 3/2019

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

WARNING: MALIGNANCIES AND RISK OF TERATOGENICITY

- **Malignancies**

Treatment with MAVENCLAD may increase the risk of malignancy. MAVENCLAD is contraindicated in patients with current malignancy. In patients with prior malignancy or with increased risk of malignancy, evaluate the benefits and risks of the use of MAVENCLAD on an individual patient basis. Follow standard cancer screening guidelines in patients treated with MAVENCLAD *[see Contraindications (4) and Warnings and Precautions (5.1)]*.

- **Risk of Teratogenicity**

MAVENCLAD is contraindicated for use in pregnant women and in women and men of reproductive potential who do not plan to use effective contraception because of the potential for fetal harm. Malformations and embryolethality occurred in animals. Exclude pregnancy before the start of treatment with MAVENCLAD in females of reproductive potential. Advise females and males of reproductive potential to use effective contraception during MAVENCLAD dosing and for 6 months after the last dose in each treatment course. Stop MAVENCLAD if the patient becomes pregnant *[see Contraindications (4), Warnings and Precautions (5.2), and Use in Specific Populations (8.1, 8.3)]*.

1 INDICATIONS AND USAGE

MAVENCLAD is indicated for the treatment of relapsing forms of multiple sclerosis (MS), to include relapsing-remitting disease and active secondary progressive disease, in adults. Because of its safety profile, use of MAVENCLAD is generally recommended for patients who have had an inadequate response to, or are unable to tolerate, an alternate drug indicated for the treatment of MS *[see Warnings and Precautions (5)]*.

Limitations of Use

MAVENCLAD is not recommended for use in patients with clinically isolated syndrome (CIS) because of its safety profile *[see Warnings and Precautions (5)]*.

2 DOSAGE AND ADMINISTRATION

2.1 Assessments Prior to Starting Each MAVENCLAD Treatment Course

Cancer Screening

Follow standard cancer screening guidelines because of the risk of malignancies *[see Boxed Warning and Warnings and Precautions (5.1)]*.

Pregnancy

Exclude pregnancy prior to treatment with MAVENCLAD in females of reproductive potential *[see Contraindications (4), Warnings and Precautions (5.2), and Use in Specific Populations (8.1, 8.3)]*.

Complete Blood Count (CBC)

Obtain a CBC with differential including lymphocyte count *[see Dosage and Administration (2.5) and Warnings and Precautions (5.3)]*. Lymphocytes must be:

- within normal limits before initiating the first treatment course
- at least 800 cells per microliter before initiating the second treatment course

If necessary, delay the second treatment course for up to 6 months to allow for recovery of lymphocytes to at least 800 cells per microliter. If this recovery takes more than 6 months, the patient should not receive further treatment with MAVENCLAD.

Infections *[see Warnings and Precautions (5.4)]*

- Exclude HIV infection.
- Perform tuberculosis screening.
- Screen for hepatitis B and C.
- Evaluate for acute infection. Consider a delay in MAVENCLAD treatment until any acute infection is fully controlled.
- Vaccination of patients who are antibody-negative for varicella zoster virus is recommended prior to initiation of MAVENCLAD.
- Administer all immunizations according to immunization guidelines prior to starting MAVENCLAD. Administer live-attenuated or live vaccines at least 4 to 6 weeks prior to starting MAVENCLAD.
- Obtain a baseline (within 3 months) magnetic resonance imaging prior to the first treatment course because of the risk of progressive multifocal leukoencephalopathy (PML).

Liver Injury

Obtain serum aminotransferase, alkaline phosphatase, and total bilirubin levels *[see Warnings and Precautions (5.7)]*.

2.2 Recommended Dosage

The recommended cumulative dosage of MAVENCLAD is 3.5 mg per kg body weight administered orally and divided into 2 yearly treatment courses (1.75 mg per kg per treatment course) (see Table 1). Each treatment course is divided into 2 treatment cycles:

Administration of First Treatment Course

- First Course/First Cycle: start any time.
- First Course/Second Cycle: administer 23 to 27 days after the last dose of First Course/First Cycle.

Administration of Second Treatment Course

- Second Course/First Cycle: administer at least 43 weeks after the last dose of First Course/Second Cycle.
- Second Course/Second Cycle: administer 23 to 27 days after the last dose of Second Course/First Cycle.

Table 1 Dose of MAVENCLAD per Cycle by Patient Weight in Each Treatment Course

Weight Range	**Dose in mg (Number of 10 mg Tablets) per Cycle**	
kg	First Cycle	Second Cycle
40* to less than 50	40 mg (4 tablets)	40 mg (4 tablets)
50 to less than 60	50 mg (5 tablets)	50 mg (5 tablets)
60 to less than 70	60 mg (6 tablets)	60 mg (6 tablets)
70 to less than 80	70 mg (7 tablets)	70 mg (7 tablets)
80 to less than 90	80 mg (8 tablets)	70 mg (7 tablets)
90 to less than 100	90 mg (9 tablets)	80 mg (8 tablets)
100 to less than 110	100 mg (10 tablets)	90 mg (9 tablets)
110 and above	100 mg (10 tablets)	100 mg (10 tablets)

*The use of MAVENCLAD in patients weighing less than 40 kg has not been investigated.

Administer the cycle dosage as 1 or 2 tablets once daily over 4 or 5 consecutive days *[see How Supplied/Storage and Handling (16.1)]*. Do not administer more than 2 tablets daily.

Following the administration of 2 treatment courses, do not administer additional MAVENCLAD treatment during the next 2 years. Treatment during these 2 years may further increase the risk of malignancy *[see Warnings and Precautions (5.1)]*. The safety and efficacy of reinitiating MAVENCLAD more than 2 years after completing 2 treatment courses has not been studied.

2.3 Missed Dose

If a dose is missed, patients should not take double or extra doses.

If a dose is not taken on the scheduled day, then the patient must take the missed dose on the following day and extend the number of days in that treatment cycle. If two consecutive doses are missed, the treatment cycle is extended by 2 days.

2.4 Administration

MAVENCLAD tablets are taken orally, with water, and swallowed whole without chewing. MAVENCLAD can be taken with or without food.

Separate administration of MAVENCLAD and any other oral drugs by at least 3 hours during the 4 to 5 day MAVENCLAD treatment cycles *[see Clinical Pharmacology (12.6)]*.

MAVENCLAD is a cytotoxic drug. Follow applicable special handling and disposal procedures *[see References (15)]*. MAVENCLAD is an uncoated tablet and must be swallowed immediately once removed from the blister. If a tablet is left on a surface, or if a broken or fragmented tablet is released from the blister, the area must be thoroughly washed with water.

The patient's hands must be dry when handling the tablets and washed thoroughly afterwards. Avoid prolonged contact with skin.

2.5 Laboratory Testing and Monitoring to Assess Safety

Cancer Screening

Follow standard cancer screening guidelines in patients treated with MAVENCLAD *[see Dosage and Administration (2.1) and Warnings and Precautions (5.1)]*.

Complete Blood Count

Obtain complete blood count (CBC) with differential including lymphocyte count:

- before initiating the first treatment course of MAVENCLAD
- before initiating the second treatment course of MAVENCLAD
- 2 and 6 months after start of treatment in each treatment course; if the lymphocyte count at month 2 is below 200 cells per microliter, monitor monthly until month 6. *See Warnings and Precautions (5.3, 5.4)* for instructions based on the patient's lymphocyte counts and clinical status (e.g., infections). Hold MAVENCLAD therapy if the lymphocyte count is below 200 cells per microliter
- periodically thereafter and when clinically indicated *[see Warnings and Precautions (5.5)]*

2.6 Recommended Concomitant Medication

Herpes Prophylaxis

Administer anti-herpes prophylaxis in patients with lymphocyte counts less than 200 cells per microliter *[see Warnings and Precautions (5.4)]*.

3 DOSAGE FORMS AND STRENGTHS

MAVENCLAD is available as 10 mg tablets. The tablets are uncoated, white, round, biconvex, and engraved with a "C" on one side and "10" on the other side.

4 CONTRAINDICATIONS

MAVENCLAD is contraindicated:

- in patients with current malignancy *[see Warnings and Precautions (5.1)]*.
- in pregnant women and in women and men of reproductive potential who do not plan to use effective contraception during MAVENCLAD dosing and for 6 months after the last dose in each treatment course. May cause fetal harm *[see Warnings and Precautions (5.2) and Use in Specific Populations (8.1, 8.3)]*.
- in patients infected with the human immunodeficiency virus (HIV) *[see Warnings and Precautions (5.4)]*.
- in patients with active chronic infections (e.g., hepatitis or tuberculosis) *[see Warnings and Precautions (5.4)]*.
- in patients with a history of hypersensitivity to cladribine *[see Warnings and Precautions (5.8)]*.

- in women intending to breastfeed on a MAVENCLAD treatment day and for 10 days after the last dose *[see Use in Specific Populations (8.2)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Malignancies

Treatment with MAVENCLAD may increase the risk of malignancy. In controlled and extension clinical studies worldwide, malignancies occurred more frequently in MAVENCLAD-treated patients [10 events in 3,754 patient-years (0.27 events per 100 patient-years)], compared to placebo patients [3 events in 2,275 patient-years (0.13 events per 100 patient-years)]. Malignancy cases in MAVENCLAD patients included metastatic pancreatic carcinoma, malignant melanoma (2 cases), ovarian cancer, compared to malignancy cases in placebo patients, all of which were curable by surgical resection [basal cell carcinoma, cervical carcinoma in situ (2 cases)]. The incidence of malignancies in United States MAVENCLAD clinical study patients was higher than the rest of the world [4 events in 189 patient-years (2.21 events per 100 patient-years) compared to 0 events in United States placebo patients]; however, the United States results were based on a limited amount of patient data.

After the completion of 2 treatment courses, do not administer additional MAVENCLAD treatment during the next 2 years *[see Dosage and Administration (2.2)]*. In clinical studies, patients who received additional MAVENCLAD treatment within 2 years after the first 2 treatment courses had an increased incidence of malignancy [7 events in 790 patient-years (0.91 events per 100 patient-years) calculated from the start of cladribine treatment in Year 3]. The risk of malignancy with reinitiating MAVENCLAD more than 2 years after the completion of 2 treatment courses has not been studied.

MAVENCLAD is contraindicated in patients with current malignancy. In patients with prior malignancy or with increased risk of malignancy, evaluate the benefits and risks of the use of MAVENCLAD on an individual patient basis. Follow standard cancer screening guidelines in patients treated with MAVENCLAD.

5.2 Risk of Teratogenicity

MAVENCLAD may cause fetal harm when administered to pregnant women. Malformations and embryolethality occurred in animals *[see Use in Specific Populations (8.1)]*. Advise women of the potential risk to a fetus during MAVENCLAD dosing and for 6 months after the last dose in each treatment course.

In females of reproductive potential, pregnancy should be excluded before initiation of each treatment course of MAVENCLAD and prevented by the use of effective contraception during MAVENCLAD dosing and for at least 6 months after the last dose of each treatment course. Women who become pregnant during treatment with MAVENCLAD should discontinue treatment *[see Use in Specific Populations (8.1, 8.3)]*. MAVENCLAD is contraindicated for use in pregnant women and in women and men of reproductive potential who do not plan to use effective contraception.

5.3 Lymphopenia

MAVENCLAD causes a dose-dependent reduction in lymphocyte count. In clinical studies, 87% of MAVENCLAD-treated patients experienced lymphopenia. The lowest absolute lymphocyte counts occurred approximately 2 to 3 months after the start of each treatment course and were lower with each additional treatment course. In patients treated with a cumulative dose of MAVENCLAD 3.5 mg per kg over 2 courses as monotherapy, 26% and 1% had nadir absolute lymphocyte counts less than 500 and less than 200 cells per microliter, respectively. At the end of the second treatment course, 2% of clinical study patients had lymphocyte counts less than 500 cells per microliter; median time to recovery to at least 800 cells per microliter was approximately 28 weeks.

Additive hematological adverse reactions may be expected if MAVENCLAD is administered prior to or concomitantly with other drugs that affect the hematological profile *[see Drug Interactions (7.3)]*. The incidence of lymphopenia less than 500 cells per microliter was higher in patients who had used drugs to treat relapsing forms of MS prior to study entry (32.1%), compared to those with no prior use of these drugs (23.8%).

Obtain complete blood count (CBC) with differential including lymphocyte count prior to, during, and after treatment with MAVENCLAD. *See Dosage and Administration (2.1, 2.5) and Warnings and Precautions (5.4)* for timing of CBC measurements and additional instructions based on the patient's lymphocyte counts and clinical status (e.g., infections).

5.4 Infections

MAVENCLAD can reduce the body's immune defense and may increase the likelihood of infections. Infections occurred in 49% of MAVENCLAD-treated patients compared to 44% of placebo patients in clinical studies. The most frequent serious infections in MAVENCLAD-treated patients included herpes zoster and pyelonephritis *(see Herpes Virus Infections)*. Fungal infections were observed, including cases of coccidioidomycosis.

HIV infection, active tuberculosis, and active hepatitis must be excluded before initiation of each treatment course of MAVENCLAD *[see Contraindications (4)]*.

Consider a delay in initiation of MAVENCLAD in patients with an acute infection until the infection is fully controlled.

Initiation of MAVENCLAD in patients currently receiving immunosuppressive or myelosuppressive therapy is not recommended *[see Drug Interactions (7.1)]*. Concomitant use of MAVENCLAD with these therapies could increase the risk of immunosuppression.

Tuberculosis

Three of 1,976 (0.2%) cladribine-treated patients in the clinical program developed tuberculosis. All three cases occurred in regions where tuberculosis is endemic. One case of tuberculosis was fatal, and two cases resolved with treatment.

Perform tuberculosis screening prior to initiation of the first and second treatment course of MAVENCLAD. Latent tuberculosis infections may be activated with use of MAVENCLAD. In patients with tuberculosis infection, delay initiation of MAVENCLAD until the infection has been adequately treated.

Hepatitis

One clinical study patient died from fulminant hepatitis B infection. Perform screening for hepatitis B and C prior to initiation of the first and second treatment course of MAVENCLAD. Latent hepatitis infections may be activated with use of MAVENCLAD. Patients who are carriers of hepatitis B or C virus may be at risk of irreversible liver damage caused by virus reactivation. In patients with hepatitis infection, delay initiation of MAVENCLAD until the infection has been adequately treated.

Herpes Virus Infections

In controlled clinical studies, 6% of MAVENCLAD-treated patients developed a herpes viral infection compared to 2% of placebo patients. The most frequent types of herpes viral infections were herpes zoster infections (2.0% vs. 0.2%) and oral herpes (2.6% vs. 1.2%). Serious herpes zoster infections occurred in 0.2% of MAVENCLAD-treated patients.

Vaccination of patients who are antibody-negative for varicella zoster virus is recommended prior to initiation of MAVENCLAD. Administer live-attenuated or live vaccines at least 4 to 6 weeks prior to starting MAVENCLAD.

The incidence of herpes zoster was higher during the period of absolute lymphocyte count less than 500 cells per microliter, compared to the time when the patients were not experiencing this degree of lymphopenia. Administer anti-herpes prophylaxis in patients with lymphocyte counts less than 200 cells per microliter.

Patients with lymphocyte counts below 500 cells per microliter should be monitored for signs and symptoms suggestive of infections, including herpes infections. If such signs and symptoms occur, initiate treatment as clinically indicated. Consider interruption or delay of MAVENCLAD until resolution of the infection.

Progressive Multifocal Leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is an opportunistic viral infection of the brain caused by the JC virus (JCV) that typically only occurs in patients who are immunocompromised, and that usually leads to death or severe disability. Typical symptoms associated with PML are diverse, progress over days to weeks, and include progressive weakness on one side of the body or clumsiness of limbs, disturbance of vision, and changes in thinking, memory, and orientation leading to confusion and personality changes.

No case of PML has been reported in clinical studies of cladribine in patients with multiple sclerosis. In patients treated with parenteral cladribine for oncologic indications, cases of PML have been reported in the postmarketing setting.

Obtain a baseline (within 3 months) magnetic resonance imaging (MRI) before initiating the first treatment course of MAVENCLAD. At the first sign or symptom suggestive of PML, withhold MAVENCLAD and perform an appropriate diagnostic evaluation. MRI findings may be apparent before clinical signs or symptoms.

Vaccinations

Administer all immunizations according to immunization guidelines prior to starting MAVENCLAD. Administer live-attenuated or live vaccines at least 4 to 6 weeks prior to starting MAVENCLAD, because of a risk of active vaccine infection *(see Herpes Virus Infections)*. Avoid vaccination with live-attenuated or live vaccines during and after MAVENCLAD treatment while the patient's white blood cell counts are not within normal limits.

5.5 Hematologic Toxicity

In addition to lymphopenia *[see Warnings and Precautions (5.3)]*, decreases in other blood cells and hematological parameters have been reported with MAVENCLAD in clinical studies. Mild to moderate decreases in neutrophil counts (cell count between 1,000 cells per microliter and < lower limit of normal (LLN)) were observed in 27% of MAVENCLAD-treated patients, compared to 13% of placebo patients whereas severe decreases in neutrophil counts (cell count below 1,000 cells per microliter) were observed in 3.6% of MAVENCLAD-treated patients, compared to 2.8% of placebo patients. Decreases in hemoglobin levels, in general mild to moderate (hemoglobin 8.0 g per dL to < LLN), were observed in 26% of MAVENCLAD-treated patients, compared to 19% of placebo patients. Decreases in platelet counts were generally mild (cell count 75,000 cells per microliter to < LLN) and were observed in 11% of MAVENCLAD-treated patients, compared to 4% of placebo patients.

In clinical studies at dosages similar to or higher than the approved MAVENCLAD dosage, serious cases of thrombocytopenia, neutropenia, and pancytopenia (some with documented bone marrow hypoplasia) requiring transfusion and granulocyte-colony stimulating factor treatment have been reported *[see Warnings and Precautions (5.6)* for information regarding graft-versus-host disease with blood transfusion*]*.

Obtain complete blood count (CBC) with differential prior to, during, and after treatment with MAVENCLAD *[see Dosage and Administration (2.1, 2.5)]*.

5.6 Graft-Versus-Host Disease With Blood Transfusion

Transfusion-associated graft-versus-host disease has been observed rarely after transfusion of nonirradiated blood in patients treated with cladribine for non-MS treatment indications.

In patients who require blood transfusion, irradiation of cellular blood components is recommended prior to administration to decrease the risk of transfusion-related graft-versus-host disease. Consultation with a hematologist is advised.

5.7 Liver Injury

In clinical studies, 0.3% of MAVENCLAD-treated patients had liver injury (serious or causing treatment discontinuation) considered related to treatment, compared to 0 placebo patients. Onset has ranged from a few weeks to several months after initiation of treatment with MAVENCLAD. Signs and symptoms of liver injury, including elevation of serum aminotransferases to greater than 20-fold the upper limit of normal, have been observed. These abnormalities resolved upon treatment discontinuation.

Obtain serum aminotransferase, alkaline phosphatase, and total bilirubin levels prior to the first and second treatment course *[see Dosage and Administration (2.1)]*. If a patient develops clinical signs, including unexplained liver enzyme elevations or symptoms suggestive of hepatic dysfunction (e.g., unexplained nausea, vomiting, abdominal pain, fatigue, anorexia, or jaundice and/or dark urine), promptly measure serum transaminases and total bilirubin and interrupt or discontinue treatment with MAVENCLAD, as appropriate.

5.8 Hypersensitivity

In clinical studies, 11% of MAVENCLAD-treated patients had hypersensitivity reactions, compared to 7% of placebo patients. Hypersensitivity reactions that were serious and/or led to discontinuation of MAVENCLAD (e.g., dermatitis, pruritis) occurred in 0.5% of MAVENCLAD-treated patients, compared to 0.1% of placebo patients. One patient had a serious hypersensitivity reaction with rash, mucous membrane ulceration, throat swelling, vertigo, diplopia, and headache after the first dose of MAVENCLAD.

If a hypersensitivity reaction is suspected, discontinue MAVENCLAD therapy. Do not use MAVENCLAD in patients with a history of hypersensitivity to cladribine *[see Contraindications (4)]*.

5.9 Cardiac Failure

In clinical studies, one MAVENCLAD-treated patient experienced life-threatening acute cardiac failure with myocarditis, which improved after approximately one week. Cases of cardiac failure have also been reported with parenteral cladribine used for treatment indications other than multiple sclerosis.

Instruct patients to seek medical advice if they experience symptoms of cardiac failure (e.g., shortness of breath, rapid or irregular heartbeat, swelling).

6 ADVERSE REACTIONS

The following serious adverse reactions and potential risks are discussed, or discussed in greater detail, in other sections of the labeling:

- Malignancies *[see Warnings and Precautions (5.1)]*
- Risk of Teratogenicity *[see Warnings and Precautions (5.2)]*
- Lymphopenia *[see Warnings and Precautions (5.3)]*
- Infections *[see Warnings and Precautions (5.4)]*
- Hematologic Toxicity *[see Warnings and Precautions (5.5)]*
- Graft-Versus-Host Disease With Blood Transfusion *[see Warnings and Precautions (5.6)]*
- Liver Injury *[see Warnings and Precautions (5.7)]*
- Hypersensitivity *[see Warnings and Precautions (5.8)]*
- Cardiac Failure *[see Warnings and Precautions (5.9)]*

6.1 Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical studies of another drug and may not reflect the rates observed in practice.

In the clinical trial program of cladribine in MS, 1,976 patients received cladribine for a total of 9,509 patient years. The mean time on study including follow-up was approximately 4.8 years, and approximately 24% of cladribine-treated patients had approximately 8 years of time on study including follow-up. Of these, 923 patients aged 18 to 66 years received MAVENCLAD as monotherapy at a cumulative dose of 3.5 mg per kg.

Table 2 shows adverse reactions in Study 1 *[see Clinical Studies (14)]* with an incidence of at least 5% for MAVENCLAD and higher than placebo. The most common (> 20%) adverse reactions reported in Study 1 are upper respiratory tract infection, headache, and lymphopenia.

Table 2 **Adverse Reactions in Study 1 with an Incidence of at Least 5% for MAVENCLAD and Higher than Placebo**

	MAVENCLAD (N=440) %	Placebo (N=435) %
Upper respiratory tract infection	38	32
Headache	25	19
Lymphopenia	24	2
Nausea	10	9
Back pain	8	6
Arthralgia and arthritis	7	5
Insomnia	6	4
Bronchitis	5	3
Hypertension	5	3
Fever	5	3
Depression	5	3

Hypersensitivity
In clinical studies, 11% of MAVENCLAD patients had hypersensitivity adverse reactions, compared to 7% of placebo patients *[see Warnings and Precautions (5.8)]*.

Alopecia
Alopecia occurred in 3% of MAVENCLAD-treated patients compared to 1% of placebo patients.

Myelodysplastic Syndrome
Cases of myelodysplastic syndrome have been reported in patients that had received parenteral cladribine at a higher dosage than that approved for MAVENCLAD. These cases occurred several years after treatment.

Herpes Meningoencephalitis
Fatal herpes meningoencephalitis occurred in one MAVENCLAD-treated patient, at a higher dosage and longer duration of therapy than the approved MAVENCLAD dosage and in combination with interferon beta-1a treatment.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)
SJS and TEN are identified risks of parenteral cladribine for the treatment of oncologic indications.

Seizures
In clinical studies, serious events of seizure occurred in 0.3% of MAVENCLAD-treated patients compared to 0 placebo patients. Serious events included generalized tonic-clonic seizures and status epilepticus. It is unknown whether these events were related to the effects of multiple sclerosis alone, to MAVENCLAD, or to a combination of both.

7 DRUG INTERACTIONS

Table 3 Drug Interactions with MAVENCLAD

7.1 Immunomodulatory, Immunosuppressive, or Myelosuppressive Drugs	
Clinical Impact	Concomitant use of MAVENCLAD with immunomodulatory, immunosuppressive, or myelosuppressive drugs may increase the risk of adverse reactions because of the additive effects on the immune system *[see Warnings and Precautions (5.4)]*.
Prevention or Management	Concomitant use with myelosuppressive or other immunosuppressive drugs is not recommended. Acute short-term therapy with corticosteroids can be administered. In patients who have previously been treated with immunomodulatory or immunosuppressive drugs, consider potential additive effect, the mode of action, and duration of effect of the other drugs prior to initiation of MAVENCLAD.
7.2 Interferon-Beta	
Clinical Impact	Concomitant use of MAVENCLAD with interferon-beta did not change the exposure of cladribine to a clinically significant effect; however, lymphopenia risk may be increased *[see Warnings and Precautions (5.3)]*.
Prevention or Management	Concomitant use is not recommended.
7.3 Hematotoxic Drugs	
Clinical Impact	Concomitant use of MAVENCLAD with hematotoxic drugs may increase the risk of adverse reactions because of the additive hematological effects *[see Warnings and Precautions (5.5)]*.
Prevention or Management	Monitor hematological parameters.
7.4 Antiviral and Antiretroviral Drugs	
Clinical Impact	Compounds that require intracellular phosphorylation to become active (e.g., lamivudine, zalcitabine, ribavirin, stavudine, and zidovudine) could interfere with the intracellular phosphorylation and activity of cladribine.
Prevention or Management	Avoid concomitant use.

7.5 Potent ENT, CNT and BCRP Transporter Inhibitors	
Clinical Impact	Cladribine is a substrate of breast cancer resistance protein (BCRP), equilibrative nucleoside (ENT1), and concentrative nucleoside (CNT3) transport proteins. The bioavailability, intracellular distribution, and renal elimination of cladribine may be altered by potent ENT1, CNT3, and BCRP transporter inhibitors.
Prevention or Management	Avoid co-administration of potent ENT1, CNT3, or BCRP transporter inhibitors (e.g., ritonavir, eltrombopag, curcumin, cyclosporine, dilazep, nifedipine, nimodipine, cilostazol, sulindac, dipyridamole, or reserpine) during the 4 to 5 day MAVENCLAD treatment cycles. If this is not possible, consider selection of alternative concomitant drugs with no or minimal ENT1, CNT3, or BCRP transporter inhibiting properties. If this is not possible, dose reduction to the minimum mandatory dose of drugs containing these compounds, separation in the timing of administration, and careful patient monitoring is recommended.
7.6 Potent BCRP and P-gp Transporter Inducers	
Clinical Impact	Possible decrease in cladribine exposure if potent BCRP or P-gp transporter inducers are co-administered.
Prevention or Management	Consider a possible decrease in cladribine efficacy if potent BCRP (e.g., corticosteroids) or P-gp (e.g., rifampicin, St. John's Wort) transporter inducers are co-administered.
7.7 Hormonal Contraceptives	
Clinical Impact	It is currently unknown whether MAVENCLAD may reduce the effectiveness of systemically acting hormonal contraceptives.
Prevention or Management	Women using systemically acting hormonal contraceptives should add a barrier method during MAVENCLAD dosing and for at least 4 weeks after the last dose in each treatment course.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

MAVENCLAD is contraindicated in pregnant women and in females and males of reproductive potential who do not plan to use effective contraception. There are no adequate data on the developmental risk associated with use of MAVENCLAD in pregnant women. Cladribine was embryolethal when administered to pregnant mice and produced malformations in mice and rabbits *[see Data]*. The observed developmental effects are consistent with the effects of cladribine on DNA *[see Contraindications (4) and Warnings and Precautions (5.2)]*.

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. The background risk of major birth defects and miscarriage for the indicated population is unknown.

Data

Animal Data
When cladribine was administered intravenously (0, 0.5, 1.5, or 3 mg/kg/day) to pregnant mice during the period of organogenesis, fetal growth retardation and malformations (including exencephaly and cleft palate) and embryofetal death were observed at the highest dose tested. An increase in skeletal variations was observed at all but the lowest dose tested. There was no evidence of maternal toxicity.

When cladribine was administered intravenously (0, 0.3, 1, and 3 mg/kg/day) to pregnant rabbits during the period of organogenesis, fetal growth retardation and a high incidence of craniofacial and limb malformations were observed at the highest dose tested, in the absence of maternal toxicity.

When cladribine was administered intravenously (0, 0.5, 1.5, or 3.0 mg/kg/day) to mice throughout pregnancy and lactation, skeletal anomalies and embryolethality were observed at all but the lowest dose tested.

8.2 Lactation

Risk Summary

MAVENCLAD is contraindicated in breastfeeding women because of the potential for serious adverse reactions in breastfed infants *[see Contraindications (4) and Warnings and Precautions (5)]*. Advise women not to breastfeed during dosing with MAVENCLAD and for 10 days after the last dose.

There are no data on the presence of cladribine in human milk, the effects on the breastfed infant, or the effects of the drug on milk production.

8.3 Females and Males of Reproductive Potential

Pregnancy Testing

In females of reproductive potential, pregnancy should be excluded before the initiation of each treatment course of MAVENCLAD *[see Use in Specific Populations (8.1)]*.

Contraception

Females
Females of reproductive potential should prevent pregnancy by use of effective contraception during MAVENCLAD dosing and for at least 6 months after the last dose in each treatment course. It is unknown if MAVENCLAD may reduce the effectiveness of the systemically acting hormonal contraceptives. Women using systemically acting hormonal contraceptives should add a barrier method during MAVENCLAD dosing and for at least 4 weeks after the last dose in each treatment course. Women who become pregnant during MAVENCLAD therapy should discontinue treatment *[see Warnings and Precautions (5.2) and Drug Interactions (7.7)]*.

Males
As cladribine interferes with DNA synthesis, adverse effects on human gametogenesis could be expected. Therefore, male patients of reproductive potential should take precautions to prevent pregnancy of their partner during MAVENCLAD dosing and for at least 6 months after the last dose in each treatment course *[see Warnings and Precautions (5.2) and Nonclinical Toxicology (13.1)]*.

8.4 Pediatric Use

The safety and effectiveness in pediatric patients (below 18 years of age) have not been established. Use of MAVENCLAD is not recommended in pediatric patients because of the risk of malignancies *[see Warnings and Precautions (5.1)]*.

8.5 Geriatric Use

Clinical studies with MAVENCLAD did not include sufficient numbers of patients aged 65 and over to determine whether they respond differently from younger patients. Other reported clinical experience has not identified differences in responses between the elderly and younger patients. Caution is recommended when MAVENCLAD is used in elderly patients, taking into account the potential greater frequency of decreased hepatic, renal, or cardiac function, concomitant diseases, and other drug therapy.

8.6 Patients with Renal Impairment

The concentration of cladribine is predicted to increase in patients with renal impairment *[see Clinical Pharmacology (12.3)]*. No dosage adjustment is recommended in patients with mild renal impairment (creatinine clearance 60 to 89 mL per minute). MAVENCLAD is not recommended in patients with moderate to severe renal impairment (creatinine clearance below 60 mL per minute) *[see Clinical Pharmacology (12.3)]*.

8.7 Patients with Hepatic Impairment

The effect of hepatic impairment on the pharmacokinetics of cladribine is unknown *[see Clinical Pharmacology (12.3)]*. No dosage adjustment is recommended in patients with mild hepatic impairment. MAVENCLAD is not recommended in patients with moderate to severe hepatic impairment (Child-Pugh score greater than 6) *[see Clinical Pharmacology (12.3)]*.

10 OVERDOSAGE

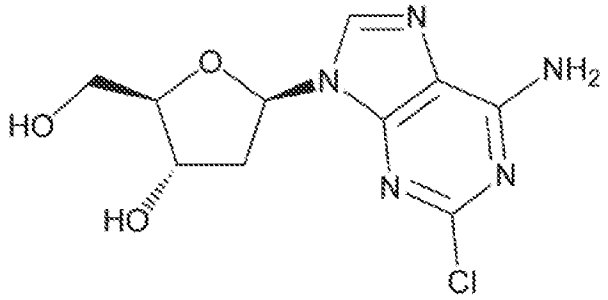
There is no experience with overdose of MAVENCLAD. Lymphopenia is known to be dose-dependent. Particularly close monitoring of hematological parameters is recommended in patients who have been exposed to an overdose of MAVENCLAD *[see Warnings and Precautions (5.3, 5.5)]*.

There is no known specific antidote to an overdose of MAVENCLAD. Treatment consists of careful observation and initiation of appropriate supportive measures. Discontinuation of MAVENCLAD may need to be considered. Because of the rapid and extensive intracellular and tissue distribution, hemodialysis is unlikely to eliminate cladribine to a significant extent.

11 DESCRIPTION

MAVENCLAD contains the nucleoside metabolic inhibitor cladribine, which is a white or almost white, non-hydroscopic, crystalline powder with the molecular formula $C_{10}H_{12}ClN_5O_3$ and molecular weight 285.69. It differs in structure from the naturally occurring nucleoside, deoxyadenosine, by the substitution of chlorine for hydrogen in the 2-position of the purine ring.

The chemical name of cladribine is 2-chloro-2′-deoxy-adenosine. The structural formula is shown below:



Cladribine is stable at slightly basic and at neutral pH. The main degradation pathway is hydrolysis and at acidic pH significant decomposition occurs with time. The ionization behavior of the molecule over the pH range 0 to 12 is characterized by a single pKa of approximately 1.21.

MAVENCLAD is provided as 10 mg tablets for oral use. Each MAVENCLAD 10 mg tablet contains cladribine as an active ingredient and hydroxypropyl betadex, magnesium stearate, and sorbitol as inactive ingredients.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The mechanism by which cladribine exerts its therapeutic effects in patients with multiple sclerosis has not been fully elucidated but is thought to involve cytotoxic effects on B and T lymphocytes through impairment of DNA synthesis, resulting in depletion of lymphocytes.

12.2 Pharmacodynamics

MAVENCLAD causes a dose-dependent reduction in lymphocyte count. The lowest absolute lymphocyte counts occurred approximately 2 to 3 months after the start of each treatment cycle and were lower with each additional treatment cycle. At the end of Year 2, 2% of patients continued to have absolute lymphocyte counts less than 500 cells per microliter. The median time to recovery from lymphocyte counts less than 500 cells per microliter to at least 800 cells per microliter was approximately 28 weeks *[see Warnings and Precautions (5.3)]*.

12.3 Pharmacokinetics

Cladribine is a prodrug that becomes active upon phosphorylation to its 2-chlorodeoxyadenosine triphosphate (Cd-ATP) metabolite.

The pharmacokinetic parameters presented below were assessed following oral administration of cladribine 10 mg, unless otherwise specified. The cladribine mean maximum concentration (C_{max}) was in the range of 22 to 29 ng/ mL and corresponding mean AUC was in the range of 80 to 101 ng•h/mL.

The C_{max} and AUC of cladribine increased proportionally across a dose range from 3 to 20 mg.

No accumulation of cladribine concentration in plasma was observed after repeated dosing.

Absorption

The bioavailability of cladribine was approximately 40%. Following fasted administration of cladribine, the median time to maximum concentration (T_{max}) was 0.5 h (range 0.5 to 1.5 hours).

Effect of Food
Following administration of cladribine with a high fat meal, the geometric mean C_{max} decreased by 29% and AUC was unchanged. The T_{max} was prolonged to 1.5 hours (range 1 to 3 hours). This difference is not expected to be clinically significant.

Distribution

Cladribine mean apparent volume of distribution ranges from 480 to 490 liters. The plasma protein binding of cladribine is 20% and is independent of concentration, in vitro.

Intracellular concentrations of cladribine and/or its metabolites in human lymphocytes were approximately 30 to 40 times extracellular, in vitro.

Cladribine has the potential to penetrate the blood brain barrier. A cerebrospinal fluid/plasma concentration ratio of approximately 0.25 was observed in cancer patients.

Elimination

Cladribine estimated terminal half-life is approximately 1 day. The intracellular half-life of the cladribine phosphorylated metabolites cladribine monophosphate (Cd-AMP) is 15 hours and Cd-ATP is 10 hours. Cladribine estimated median apparent renal clearance is 22.2 liter per hour and non-renal clearance is 23.4 liter per hour.

Metabolism
Cladribine is a prodrug that is phosphorylated to Cd-AMP by deoxycytidine kinase (and also by deoxyguanosine kinase in the mitochondria) in lymphocytes. Cd-AMP is further phosphorylated to cladribine diphosphate (Cd-ADP) and the active moiety Cd-ATP. The dephosphorylation and deactivation of Cd-AMP is catalyzed by cytoplasmic 5'-nucleotidase (5'-NTase).

The metabolism of cladribine in whole blood has not been fully characterized. However, extensive whole blood and negligible hepatic enzyme metabolism was observed, in vitro.

Excretion
After administration of 10 mg oral cladribine in MS patients, 28.5 [20] (mean [SD]) percent of the dose was excreted unchanged via the renal route. Renal clearance exceeded the glomerular filtration rate, indicating active renal secretion of cladribine.

Specific Populations

No studies have been conducted to evaluate the pharmacokinetics of cladribine in elderly or in patients with renal or hepatic impairment.

There were no clinically significant differences in the pharmacokinetics of cladribine based on age (range 18 to 65 years) or gender. The effect of hepatic impairment on the pharmacokinetics of cladribine is unknown.

Patients with Renal Impairment
Renal clearance of cladribine was shown to be dependent on creatinine clearance (CL_{CR}). No dedicated studies have been conducted in patients with renal impairment, however patients with mild renal impairment (CL_{CR} of 60 mL to below 90 mL per minute) were included in Study 1. A pooled pharmacokinetic analysis estimated a decrease of 18% in total clearance in a typical subject with a CL_{CR} of 65 mL per minute leading to an increase in cladribine exposure of 25%. Clinical experience in patients with moderate to severe renal impairment (i.e., CL_{CR} below 60 mL per minute) is limited *[see Use in Specific Populations (8.6)]*.

Drug Interaction Studies

Clinical Studies
No clinically significant differences in cladribine pharmacokinetics were observed when used concomitantly with pantoprazole or interferon beta-1a.

In Vitro Studies
It has been reported that lamivudine can inhibit the phosphorylation of cladribine intracellularly. Potential competition for intracellular phosphorylation exists between cladribine and compounds that require intracellular phosphorylation to become active (e.g., lamivudine, zalcitabine, ribavirin, stavudine, and zidovudine).

Cytochrome P450 (CYP) Enzymes: Cladribine is not a substrate of cytochrome P450 enzymes and does not show significant potential to act as inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. Cladribine has no clinically meaningful inductive effect on CYP1A2, CYP2B6 and CYP3A4 enzymes.

Transporter Systems: Cladribine is a substrate of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), equilibrative nucleoside transporter 1 (ENT1) and concentrative nucleoside transporter 3 (CNT3). Inhibition of BCRP in the gastrointestinal tract may increase the oral bioavailability and systemic exposure of cladribine. Intracellular distribution and renal elimination of cladribine may be altered by potent ENT1, CNT3 transporter inhibitors.

12.6 Hydroxypropyl Betadex-Related Complex Formation

MAVENCLAD contains hydroxypropyl betadex that may be available for complex formation with the active ingredients of other drugs. Complex formation between free hydroxypropyl betadex, released from the cladribine tablet formulation, and concomitant ibuprofen, furosemide, and gabapentin was observed. Concomitant use with MAVENCLAD may increase the bioavailability of other drugs (especially agents with low solubility), which may increase the risk or severity of adverse reactions *[see Dosage and Administration (2.4)]*.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

In mice administered cladribine (0, 0.1, 1, or 10 mg/kg) by subcutaneous injection intermittently (7 daily doses followed by 21 days of non-dosing per cycle) for 22 months, an increase in Harderian gland tumors (adenoma) was observed at the highest dose tested.

Mutagenesis

Cladribine was negative for mutagenicity in in vitro (reverse mutation in bacteria, CHO/HGPRT mammalian cell) assays.

Cladribine was positive for clastogenicity in an in vitro mammalian cell assay, in the absence and presence of metabolic activation, and in an in vivo mouse micronucleus assay.

Impairment of Fertility

When cladribine (0, 1, 5, 10, or 30 mg/kg/day) was administered by subcutaneous injection to male mice prior to and during mating to untreated females, no effects on fertility were observed. However, an increase in non-motile sperm was observed at the highest dose tested. In female mice, administration of cladribine (0, 1, 2, 4, or 8 mg/kg/day) by subcutaneous injection prior to and during mating to untreated males and continuing to gestation day 6 caused an increase in embryolethality at the highest dose tested.

In monkeys administered cladribine (0, 0.15, 0.3, or 1.0 mg/kg) by subcutaneous injection intermittently (7 consecutive daily doses followed by 21 days of non-dosing per cycle) for one year, testicular degeneration was observed at the highest dose tested.

14 CLINICAL STUDIES

The efficacy of MAVENCLAD was demonstrated in a 96-week randomized, double-blind, placebo-controlled clinical study in patients with relapsing forms of MS (Study 1; NCT00213135).

Patients were required to have at least 1 relapse in the previous 12 months. The median age was 39 years (range 18 to 65) and the female-to-male ratio was approximately 2:1. The mean duration of MS prior to study enrollment was 8.7 years, and the median baseline neurological disability based on Kurtzke Expanded Disability Status Scale (EDSS) score across all treatment groups was 3.0. Over two thirds of the study patients were treatment-naive for drugs used to treat relapsing forms of MS.

1,326 patients were randomized to receive either placebo (n = 437), or a cumulative oral dosage of MAVENCLAD 3.5 mg per kg (n = 433) or 5.25 mg per kg body weight (n = 456) over the 96-week study period in 2 treatment courses. Patients randomized to the 3.5 mg per kg cumulative dose received a first treatment course at Weeks 1 and 5 of the first year and a second treatment course at Weeks 1 and 5 of the second year *[see Dosage and Administration (2.2)]*. Patients randomized to the 5.25 mg per kg cumulative dose received additional treatment at Weeks 9 and 13 of the first year. Higher cumulative doses did not add any clinically meaningful benefit, but were associated with a higher incidence in grade 3 lymphopenia or higher (44.9% in the 5.25 mg per kg group vs. 25.6% in the 3.5 mg per kg group). Ninety-two percent of patients treated with MAVENCLAD 3.5 mg per kg and 87% of patients receiving placebo completed the full 96 weeks of the study.

The primary outcome of Study 1 was the annualized relapse rate (ARR). Additional outcome measures included the proportion of patients with confirmed disability progression, the time to first qualifying relapse, the mean number of MRI T1 Gadolinium-enhancing (Gd+) lesions, and new or enlarging MRI T2 hyperintense lesions. Disability progression was measured in terms of a 3-month sustained change in EDSS score of at least one point, if baseline EDSS score was between 0.5 and 4.5 inclusively, or at least 1.5 points if the baseline EDSS score was 0, or at least 0.5 point if the baseline EDSS score was at least 5, over a period of at least 3 months.

MAVENCLAD 3.5 mg per kg significantly lowered the annualized relapse rate. The results from Study 1 are presented in Table 4.

Table 4 Clinical Outcomes in Study 1 (96 Weeks) - Primary and Secondary Endpoints

Endpoints	MAVENCLAD Cumulative Dose 3.5 mg per kg (n = 433)	Placebo (n = 437)
Clinical Endpoints		
Annualized relapse rate (ARR)	0.14*	0.33
Relative reduction in ARR	58%	
Proportion of patients without relapse	81%**	63%
Time to 3-month confirmed EDSS progression, HR	0.67**	
Proportion of patients with 3-month EDSS progression	13%	19%
MRI Endpoints		
Median Number of Active T1 Gd+ Lesions	0*	0.33
Median Number of Active T2 Lesions	0*	0.67

* $p < 0.001$ compared to placebo
** nominal $p < 0.05$ compared to placebo
HR: Hazard Ratio

15 REFERENCES

1 "OSHA Hazardous Drugs". OSHA. http://www.osha.gov/SLTC/hazardousdrugs/index.html.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

MAVENCLAD tablets, 10 mg, are uncoated, white, round, biconvex, and engraved with a "C" on one side and "10" on the other side. Each tablet is packaged in a child-resistant day pack containing one or two tablets in a blister card.

Dispense one box for each treatment cycle with a Medication Guide *[see Dosage and Administration (2.2)]*.

Presentations

NDC 44087-400-11	Box of 1 tablet: One day pack containing one tablet.
NDC 44087-400-12	Box of 2 tablets: One day pack containing two tablets.
NDC 44087-400-04	Box of 4 tablets: Four day packs each containing one tablet.
NDC 44087-400-05	Box of 5 tablets: Five day packs each containing one tablet.
NDC 44087-400-06	Box of 6 tablets: One day pack containing two tablets. Four day packs each containing one tablet.
NDC 44087-400-07	Box of 7 tablets: Two day packs each containing two tablets. Three day packs each containing one tablet.
NDC 44087-400-08	Box of 8 tablets: Three day packs each containing two tablets. Two day packs each containing one tablet.
NDC 44087-400-09	Box of 9 tablets: Four day packs each containing two tablets. One day pack containing one tablet.
NDC 44087-400-10	Box of 10 tablets: Five day packs each containing two tablets.

16.2 Storage and Handling

Store at controlled room temperature, 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F) *[see USP Controlled Room Temperature]*. Store in original package in order to protect from moisture.

MAVENCLAD is a cytotoxic drug. Follow applicable special handling and disposal procedures *[see References (15)]*.[1]

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Medication Guide).

Malignancies

Inform patients that MAVENCLAD may increase their risk of malignancies. Instruct patients to follow standard cancer screening guidelines *[see Dosage and Administration (2) and Warnings and Precautions (5.1)]*.

Risk of Teratogenicity

Inform patients that MAVENCLAD may cause fetal harm. Discuss with women of childbearing age whether they are pregnant, might be pregnant, or are trying to become pregnant. Before initiating each treatment course, inform patients about the potential risk to the fetus, if female patients or partners of male patients get pregnant during MAVENCLAD dosing or within 6 months after the last dose in each treatment course *[see Warnings and Precautions (5.2) and Use in Specific Populations (8.1, 8.3)]*.

Instruct female patients of childbearing potential to use effective contraception during MAVENCLAD dosing and for at least 6 months after the last dose in each treatment course to avoid pregnancy. Advise women using systemically acting hormonal contraceptives to add a barrier method during MAVENCLAD dosing and for at least 4 weeks after the last dose in each treatment course because MAVENCLAD may reduce the effectiveness of the hormonal contraceptive *[see Drug Interactions (7.7)]*.

Instruct male patients to take precautions to prevent pregnancy of their partner during MAVENCLAD dosing and for at least 6 months after the last dose in each treatment course.

Advise patients that female patients or partners of male patients who get pregnant immediately inform their healthcare provider.

Lactation

Inform women that they cannot breastfeed on a MAVENCLAD treatment day and for 10 days after the last dose *[see Use in Specific Populations (8.2)]*.

Lymphopenia and Other Hematologic Toxicity

Inform patients that MAVENCLAD may decrease lymphocyte counts and may also decrease counts of other blood cells. A blood test should be obtained before starting a treatment course, 2 and 6 months after start of treatment in each treatment course, periodically thereafter, and when clinically needed. Advise patients to keep all appointments for lymphocyte monitoring during and after MAVENCLAD treatment *[see Dosage and Administration (2.5) and Warnings and Precautions (5.3, 5.5)]*.

Infections

Inform patients that use of MAVENCLAD may increase the risk of infections. Instruct patients to notify their healthcare provider promptly if fever or other signs of infection such as aching, painful muscles, headache, generally feeling unwell or loss of appetite occur while on therapy or after a course of treatment *[see Warnings and Precautions (5.4)]*.

Advise patients that PML has happened with parenteral cladribine used in oncologic indications. Inform the patient that PML is characterized by a progression of deficits and usually leads to death or severe disability over weeks or months. Instruct the patient of the importance of contacting their doctor if they develop any symptoms suggestive of PML. Inform the patient that typical symptoms associated with PML are diverse, progress over days to weeks, and include progressive weakness on one side of the body or clumsiness of limbs, disturbance of vision, and changes in thinking, memory, and orientation leading to confusion and personality changes *[see Warnings and Precautions (5.4)]*.

Liver Injury

Inform patients that MAVENCLAD may cause liver injury. Instruct patients treated with MAVENCLAD to report promptly any symptoms that may indicate liver injury, including fatigue, anorexia, right upper abdominal discomfort, dark urine, or jaundice. A blood test should be obtained prior to each treatment course with MAVENCLAD and as clinically indicated thereafter *[see Warnings and Precautions (5.7)]*.

Hypersensitivity

Advise patients to seek immediate medical attention if they experience any symptoms of serious or severe hypersensitivity reactions, including skin reactions *[see Warnings and Precautions (5.8)]*.

Cardiac Failure

Advise patients that MAVENCLAD may cause cardiac failure. Instruct patients to seek medical advice if they experience symptoms of cardiac failure (e.g., shortness of breath, rapid or irregular heartbeat, swelling) *[see Warnings and Precautions (5.9)]*.

Treatment Handling and Administration

Instruct patients that MAVENCLAD is a cytotoxic drug and to use care when handling MAVENCLAD tablets, limit direct skin contact with the tablets, and wash exposed areas thoroughly. Advise patients to keep the tablets in the original package until just prior to each scheduled dose and consult their pharmacist on the proper disposal of unused tablets *[see Dosage and Administration (2.4) and How Supplied/Storage and Handling (16.2)]*.

Distributed by:
EMD Serono, Inc.
Rockland, MA 02370

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MEDICATION GUIDE

MAVENCLAD® (MAY-ven-klad)
(cladribine)
tablets, for oral use

What is the most important information I should know about MAVENCLAD?

MAVENCLAD can cause serious side effects, including:

- **Risk of cancer (malignancies).** Treatment with MAVENCLAD may increase your risk of developing cancer. Talk to your healthcare provider about your risk of developing cancer if you receive MAVENCLAD. You should follow your healthcare provider instructions about screening for cancer.
- **MAVENCLAD may cause birth defects if used during pregnancy. Females must not be pregnant when they start treatment with MAVENCLAD or become pregnant during MAVENCLAD dosing and within 6 months after the last dose of each yearly treatment course. Stop your treatment with MAVENCLAD and call your healthcare provider right away if you become pregnant during treatment with MAVENCLAD.**
  - For females who are able to become pregnant:
    - Your healthcare provider should order a pregnancy test for you before you begin your first and second yearly treatment course of MAVENCLAD to make sure that you are not pregnant. Your healthcare provider will decide when to do the test.
    - Use effective birth control (contraception) on the days on which you take MAVENCLAD and for at least 6 months after the last dose of each yearly treatment course.
      - Talk to your healthcare provider if you use oral contraceptives (the "pill").
      - You should use a second method of birth control on the days on which you take MAVENCLAD and for at least 4 weeks after your last dose of each yearly treatment course.
  - For males with female partners who are able to become pregnant:
    - Use effective birth control (contraception) during the days on which you take MAVENCLAD and for at least 6 months after the last dose of each yearly treatment course.

What is MAVENCLAD?

MAVENCLAD is a prescription medicine used to treat relapsing forms of multiple sclerosis (MS), to include relapsing-remitting disease and active secondary progressive disease, in adults. Because of its safety profile, MAVENCLAD is generally used in people who have tried another MS medicine that they could not tolerate or that has not worked well enough.

MAVENCLAD is not recommended for use in people with clinically isolated syndrome (CIS).

It is not known if MAVENCLAD is safe and effective in children under 18 years of age.

Do not take MAVENCLAD if you:

- have cancer (malignancy).
- are pregnant, plan to become pregnant, or are a woman of childbearing age or a man able to father a child and you are not using birth control. See "**What is the most important information I should know about MAVENCLAD?**"
- are human immunodeficiency virus (HIV) positive.
- have active infections, including tuberculosis (TB), hepatitis B or C.
- are allergic to cladribine.
- are breastfeeding. See "**Before you take MAVENCLAD, tell your healthcare provider about all of your medical conditions, including if you:**"

Before you take MAVENCLAD, tell your healthcare provider about all of your medical conditions, including if you:

- think you have an infection.
- have heart failure.
- have liver or kidney problems.
- have taken, take, or plan to take medicines that affect your immune system or your blood cells, or other treatments for MS. Certain medicines can increase your risk of getting an infection.
- have had a recent vaccination or are scheduled to receive any vaccinations. You should not receive live or live-attenuated vaccines within the 4 to 6 weeks preceding your treatment with MAVENCLAD. You should not receive these types of vaccines during your treatment with MAVENCLAD and until your healthcare provider tells you that your immune system is no longer weakened.
- have or have had cancer.
- are breastfeeding or plan to breastfeed. It is not known if MAVENCLAD passes into your breast milk. Do not breastfeed on the days, on which you take MAVENCLAD, and for 10 days after the last dose. See "**Do not take**

MAVENCLAD if you:"

Tell your healthcare provider about all the medicines you take, including prescription and over-the-counter medicines, vitamins, and herbal supplements.

How should I take MAVENCLAD?

- MAVENCLAD is given as two yearly treatment courses.
- Each yearly treatment course consists of 2 treatment weeks (also called cycles) that will be about a month apart. Your healthcare provider will tell you when you have to start your treatment weeks and how many tablets per week you need, depending on your weight. Each treatment week is 4 or 5 days.
- Your pharmacist will dispense a carton of MAVENCLAD for each treatment week. The prescribed number of tablets per day are provided in child resistant day packs.
- Take MAVENCLAD exactly as your healthcare provider tells you. Do not change your dose or stop taking MAVENCLAD unless your healthcare provider tells you to.
- Take MAVENCLAD with water and swallow whole without chewing. MAVENCLAD can be taken with or without food.
- Swallow MAVENCLAD right away after opening the blister pack.
- Your hands must be dry when handling MAVENCLAD and washed well with water afterwards.
- Limit contact with your skin. Avoid touching your nose, eyes and other parts of the body. If you get MAVENCLAD on your skin or on any surface, wash it right away with water.
- Take MAVENCLAD at least 3 hours apart from other medicines taken by mouth during the 4- to 5-day MAVENCLAD treatment week.
- If you miss a dose, take it as soon as you remember on the same day. If the whole day passes before you remember, take your missed dose the next day. **Do not take 2 doses at the same time**. Instead, you will extend the number of days in that treatment week.

Your healthcare provider will continue to monitor your health during the 2 yearly treatment courses, and for at least another 2 years during which you do not need to take MAVENCLAD. It is not known if MAVENCLAD is safe and effective in people who restart MAVENCLAD treatment more than 2 years after completing 2 yearly treatment courses.

What are the possible side effects of MAVENCLAD?

MAVENCLAD can cause serious side effects, including:

- See **"What is the most important information I should know about MAVENCLAD?"**
- **low blood cell counts.** Low blood cell counts have happened and can increase your risk of infections during your treatment with MAVENCLAD. Your healthcare provider will do blood tests before you start treatment with MAVENCLAD, during your treatment with MAVENCLAD, and afterward, as needed.
- **serious infections such as:**
  - **TB, hepatitis B or C, and shingles (herpes zoster).** Fatal cases of TB and hepatitis have happened with cladribine during clinical studies. Tell your healthcare provider right away if you get any symptoms of the following infection related problems or if any of the symptoms get worse, including:
    - fever
    - aching painful muscles
    - headache
    - feeling of being generally unwell
    - loss of appetite
    - burning, tingling, numbness or itchiness of the skin in the affected area
    - skin blotches, blistered rash and severe pain
  - **progressive multifocal leukoencephalopathy (PML).** PML is a rare brain infection that usually leads to death or severe disability. Although PML has not been seen in MS patients taking MAVENCLAD, it may happen in people with weakened immune systems. Symptoms of PML get worse over days to weeks. Call your healthcare provider right away if you have any new or worsening neurologic signs or symptoms of PML, that have lasted several days, including:
    - weakness on 1 side of your body
    - loss or coordination in your arms and legs
    - decreased strength
    - problems with balance
    - changes in your vision
    - changes in your thinking or memory
    - confusion
    - changes in your personality
- **liver problems.** MAVENCLAD may cause liver problems. Your healthcare provider should do blood tests to check your liver before you start taking MAVENCLAD. Call your healthcare provider right away if you have any of the following symptoms of liver problems:
  - nausea
  - vomiting
  - stomach pain
  - tiredness
  - loss of appetite
  - your skin or the whites or your eyes turn yellow
  - dark urine

- **allergic reactions (hypersensitivities).** MAVENCLAD can cause serious allergic reactions. Stop your treatment with MAVENCLAD and go to the closest emergency room for medical help right away if you have any signs or symptoms of allergic reactions. Symptoms of an allergic reaction may include: skin rash, swelling or itching of the face, lips, tongue or throat, or trouble breathing.
- **heart failure.** MAVENCLAD may cause heart failure, which means your heart may not pump as well as it should. Call your healthcare provider or go to the closest emergency room for medical help right away if you have any signs or symptoms such as shortness of breath, a fast or irregular heart beat, or unusual swelling in your body.

Your healthcare provider may delay or completely stop treatment with MAVENCLAD if you have severe side effects.

The most common side effects of MAVENCLAD include:

- upper respiratory infection
- headache
- low white blood cell counts

These are not all the possible side effects of MAVENCLAD. Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

How should I store MAVENCLAD?

- MAVENCLAD comes in a child resistant package.
- Store MAVENCLAD at room temperature between 68°F and 77°F (20°C and 25°C).
- Store MAVENCLAD in the original package to protect from moisture.
- Ask your healthcare provider or pharmacist about how to safely throw away any unused or expired MAVENCLAD tablets and packaging.

Keep MAVENCLAD and all medicines out of the reach of children.

General information about the safe and effective use of MAVENCLAD

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide. Do not use MAVENCLAD for a condition for which it was not prescribed. Do not give MAVENCLAD to other people, even if they have the same symptoms that you have. It may harm them. You can ask your healthcare provider for information about MAVENCLAD that is written for health professionals.

What are the ingredients in MAVENCLAD?

Active ingredient: cladribine

Inactive ingredients: hydroxypropyl betadex, magnesium stearate, and sorbitol.

Distributed by: EMD Serono, Inc., Rockland, MA 02370

For more information, call toll-free 1-877-447-3243 or go to www.mavenclad.com

This Medication Guide has been approved by the U.S. Food and Drug Administration. Issued: 3/2019

EXHIBIT L

Drugs@FDA: FDA Approved Drug Products

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New Drug Application (NDA): 020229
Company: JANSSEN PHARMS

Products on NDA 020229

CSV | Excel | Print

Drug Name	Active Ingredients	Strength	Dosage Form/Route	Marketing Status	TE Code	RLD	RS
LEUSTATIN	CLADRIBINE	1MG/ML **Federal Register determination that product was not discontinued or withdrawn for safety or efficacy reasons**	INJECTABLE;INJECTION	Discontinued	None	Yes	No

Showing 1 to 1 of 1 entries

Approval Date(s) and History, Letters, Labels, Reviews for NDA 020229

Original Approvals or Tentative Approvals

CSV | Excel | Print

Action Date	Submission	Action Type	Submission Classification	Review Priority; Orphan Status	Letters, Reviews, Labels, Patient Package Insert	Notes
02/26/1993	ORIG-1	Approval	Type 1 - New Molecular Entity	STANDARD		Withdrawn FR Effective 11/03/2016 Label is not available on this site.

Showing 1 to 1 of 1 entries

Supplements

CSV | Excel | Print

Action Date	Submission	Supplement Categories or Approval Type	Letters, Reviews, Labels, Patient Package Insert
08/02/2012	SUPPL-34		**Label (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/020 Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2012/**
06/29/2006	SUPPL-30		**Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2006/**

Action Date	Submission	Supplement Categories or Approval Type	Letters, Reviews, Labels, Patient Package Insert
08/22/2002	SUPPL-21		Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2002/
08/20/2002	SUPPL-7		Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2002/
08/20/2002	SUPPL-4		Letter (PDF) (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2002/

Showing 1 to 5 of 5 entries

Labels for NDA 020229

LEUSTATIN® (cladribine)
Injection
For Intravenous Infusion Only

WARNING

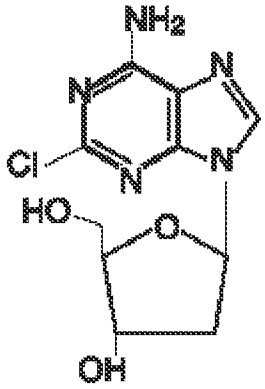
LEUSTATIN (cladribine) Injection should be administered under the supervision of a qualified physician experienced in the use of antineoplastic therapy. Suppression of bone marrow function should be anticipated. This is usually reversible and appears to be dose dependent. Serious neurological toxicity (including irreversible paraparesis and quadraparesis) has been reported in patients who received LEUSTATIN Injection by continuous infusion at high doses (4 to 9 times the recommended dose for Hairy Cell Leukemia). Neurologic toxicity appears to demonstrate a dose relationship; however, severe neurological toxicity has been reported rarely following treatment with standard cladribine dosing regimens.

Acute nephrotoxicity has been observed with high doses of LEUSTATIN (4 to 9 times the recommended dose for Hairy Cell Leukemia), especially when given concomitantly with other nephrotoxic agents/therapies.

DESCRIPTION

LEUSTATIN (cladribine) Injection (also commonly known as 2-chloro-2′-deoxy-β-D-adenosine) is a synthetic antineoplastic agent for continuous intravenous infusion. It is a clear, colorless, sterile, preservative-free, isotonic solution. LEUSTATIN Injection is available in single-use vials containing 10 mg (1 mg/mL) of cladribine, a chlorinated purine nucleoside analog. Each milliliter of LEUSTATIN Injection contains 1 mg of the active ingredient and 9 mg (0.15 mEq) of sodium chloride as an inactive ingredient. The solution has a pH range of 5.5 to 8.0. Phosphoric acid and/or dibasic sodium phosphate may have been added to adjust the pH to 6.3±0.3.

The chemical name for cladribine is 2-chloro-6-amino-9-(2-deoxy-β-D-erythropento-furanosyl) purine and the structure is represented below:



cladribine MW 285.7

CLINICAL PHARMACOLOGY

Cellular Resistance and Sensitivity:

The selective toxicity of 2-chloro-2′-deoxy-β-D-adenosine towards certain normal and malignant lymphocyte and monocyte populations is based on the relative activities of deoxycytidine kinase and deoxynucleotidase. Cladribine passively crosses the cell membrane. In cells with a high ratio of deoxycytidine kinase to deoxynucleotidase, it is phosphorylated by deoxycytidine kinase to 2-chloro-2′-deoxy- β -D-adenosine monophosphate (2-CdAMP). Since 2-chloro-2′-deoxy- β -D-adenosine is resistant to deamination by adenosine deaminase and there is little deoxynucleotide deaminase in lymphocytes and monocytes, 2-CdAMP accumulates intracellularly and is subsequently converted into the active triphosphate deoxynucleotide, 2-chloro-2′-deoxy- β -D-adenosine triphosphate (2-CdATP). It is postulated that cells with high deoxycytidine kinase and low deoxynucleotidase activities will be selectively killed by 2-chloro-2′-deoxy- β -D-adenosine as toxic deoxynucleotides accumulate intracellularly.

Cells containing high concentrations of deoxynucleotides are unable to properly repair single-strand DNA breaks. The broken ends of DNA activate the enzyme poly (ADP-ribose) polymerase resulting in NAD and ATP depletion and disruption of cellular metabolism. There is evidence, also, that 2-CdATP is incorporated into the DNA of dividing cells, resulting in impairment of DNA synthesis. Thus, 2-chloro-2′-deoxy- β -D-adenosine can be distinguished from other chemotherapeutic agents affecting purine metabolism in that it is cytotoxic to both actively dividing and quiescent lymphocytes and monocytes, inhibiting both DNA synthesis and repair.

Pharmacokinetics

In a clinical investigation, 17 patients with Hairy Cell Leukemia and normal renal function were treated for 7 days with the recommended treatment regimen of LEUSTATIN Injection (0.09 mg/kg/day) by continuous intravenous infusion. The mean steady-state serum concentration was estimated to be 5.7 ng/mL with an estimated systemic clearance of 663.5 mL/h/kg when LEUSTATIN was given by continuous infusion over 7 days. In Hairy Cell Leukemia patients, there does not appear to be a relationship between serum concentrations and ultimate clinical outcome.

In another study, 8 patients with hematologic malignancies received a two (2) hour infusion of LEUSTATIN Injection (0.12 mg/kg). The mean end-of-infusion plasma LEUSTATIN concentration was 48±19 ng/mL. For 5 of these patients, the disappearance of LEUSTATIN could be described by either a biphasic or triphasic decline. For these patients with normal renal function, the mean terminal half-life was 5.4 hours. Mean values for clearance and steady-state volume of distribution were 978±422 mL/h/kg and 4.5±2.8 L/kg, respectively.

Cladribine plasma concentration after intravenous administration declines multi-exponentially with an average half-life of 6.7 +/- 2.5 hours. In general, the apparent volume of distribution of cladribine is approximately 9 L/kg, indicating an extensive distribution in body tissues.

Cladribine penetrates into cerebrospinal fluid. One report indicates that concentrations are approximately 25% of those in plasma.

LEUSTATIN is bound approximately 20% to plasma proteins.

Except for some understanding of the mechanism of cellular toxicity, no other information is available on the metabolism of LEUSTATIN in humans. An average of 18% of the administered dose has been reported to be excreted in urine of patients with solid tumors during a 5-day continuous intravenous infusion of 3.5-8.1 mg/m^2/day of LEUSTATIN. The effect of renal and hepatic impairment on the elimination of cladribine has not been investigated in humans.

CLINICAL STUDIES

Two single-center open label studies of LEUSTATIN (cladribine) have been conducted in patients with Hairy Cell Leukemia with evidence of active disease requiring therapy. In the study conducted at the Scripps Clinic and Research Foundation (Study A), 89 patients were treated with a single course of LEUSTATIN Injection given by continuous intravenous infusion for 7 days at a dose of

0.09 mg/kg/day. In the study conducted at the M.D. Anderson Cancer Center (Study B), 35 patients were treated with a 7-day continuous intravenous infusion of LEUSTATIN Injection at a comparable dose of 3.6 mg/m^2/day. A complete response (CR) required clearing of the peripheral blood and bone marrow of hairy cells and recovery of the hemoglobin to 12 g/dL, platelet count to 100 x 10^9/L, and absolute neutrophil count to 1500 x 10^6/L. A good partial response (GPR) required the same hematologic parameters as a complete response, and that fewer than 5% hairy cells remain in the bone marrow. A partial response (PR) required that hairy cells in the bone marrow be decreased by at least 50% from baseline and the same response for hematologic parameters as for complete response. A pathologic relapse was defined as an increase in bone marrow hairy cells to 25% of pretreatment levels. A clinical relapse was defined as the recurrence of cytopenias, specifically, decreases in hemoglobin $\geq$ 2 g/dL, ANC $\geq$ 25% or platelet counts $\geq$ 50,000. Patients who met the criteria for a complete response but subsequently were found to have evidence of bone marrow hairy cells (< 25% of pretreatment levels) were reclassified as partial responses and were not considered to be complete responses with relapse.

Among patients evaluable for efficacy (N=106), using the hematologic and bone marrow response criteria described above, the complete response rates in patients treated with LEUSTATIN Injection were 65% and 68% for Study A and Study B, respectively, yielding a combined complete response rate of 66%. Overall response rates (i.e., Complete plus Good Partial plus Partial Responses) were 89% and 86% in Study A and Study B, respectively, for a combined overall response rate of 88% in evaluable patients treated with LEUSTATIN Injection.

Using an intent-to-treat analysis (N=123) and further requiring no evidence of splenomegaly as a criterion for CR (i.e., no palpable spleen on physical examination and $\leq$ 13 cm on CT scan), the complete response rates for Study A and Study B were 54% and 53%, respectively, giving a combined CR rate of 54%. The overall response rates (CR + GPR + PR) were 90% and 85%, for Studies A and B, respectively, yielding a combined overall response rate of 89%.

RESPONSE RATES TO LEUSTATIN TREATMENT IN PATIENTS WITH HAIRY CELL LEUKEMIA

	CR	Overall
Evaluable Patients N=106	66%	88%
Intent-to-treat Population N=123	54%	89%

In these studies, 60% of the patients had not received prior chemotherapy for Hairy Cell Leukemia or had undergone splenectomy as the only prior treatment and were receiving

LEUSTATIN as a first-line treatment. The remaining 40% of the patients received LEUSTATIN as a second-line treatment, having been treated previously with other agents, including α-interferon and/or deoxycoformycin. The overall response rate for patients without prior chemotherapy was 92%, compared with 84% for previously treated patients. LEUSTATIN is active in previously treated patients; however, retrospective analysis suggests that the overall response rate is decreased in patients previously treated with splenectomy or deoxycoformycin and in patients refractory to α-interferon.

OVERALL RESPONSE RATES (CR + GPR + PR) TO LEUSTATIN TREATMENT IN PATIENTS WITH HAIRY CELL LEUKEMIA

	OVERALL RESPONSE (N = 123)	NR + RELAPSE
No Prior Chemotherapy	68/74	6 + 4
	92%	14%
Any Prior Chemotherapy	41/49	8 + 3
	84%	22%
Previous Splenectomy	32/41*	9 + 1
	78%	24%
Previous Interferon	40/48	8 + 3
	83%	23%
Interferon Refractory	6/11*	5 + 2
	55%	64%
Previous Deoxycoformycin	3/6*	3 + 1
	50%	66%

NR = No Response
* $P < 0.05$

After a reversible decline, normalization of peripheral blood counts (Hemoglobin >12.0 g/dL, Platelets >100 x 10^9/L, Absolute Neutrophil Count (ANC) >1500 x 10^6/L) was achieved by 92% of evaluable patients. The median time to normalization of peripheral counts was 9 weeks from the start of treatment (Range: 2 to 72). The median time to normalization of Platelet Count was 2 weeks, the median time to normalization of ANC was 5 weeks and the median time to normalization of Hemoglobin was 8 weeks. With normalization of Platelet Count and Hemoglobin, requirements for platelet and RBC transfusions were abolished after Months 1 and 2, respectively, in those patients with complete response. Platelet recovery may be delayed in a minority of patients with severe baseline thrombocytopenia. Corresponding to normalization of ANC, a trend toward a reduced incidence of infection was seen after the third month, when compared to the months immediately preceding LEUSTATIN therapy. (see also WARNINGS, PRECAUTIONS and ADVERSE REACTIONS)

LEUSTATIN TREATMENT IN PATIENTS WITH HAIRY CELL LEUKEMIA TIME TO NORMALIZATION OF PERIPHERAL BLOOD COUNTS

Parameter	Median Time to Normalization of Count*
Platelet Count	2 weeks
Absolute Neutrophil Count	5 weeks
Hemoglobin	8 weeks
ANC, Hemoglobin and Platelet Count	9 weeks

* Day 1 = First day of infusion

For patients achieving a complete response, the median time to response (i.e., absence of hairy cells in bone marrow and peripheral blood together with normalization of peripheral blood parameters), measured from treatment start, was approximately 4 months. Since bone marrow aspiration and biopsy were frequently not performed at the time of peripheral blood normalization, the median time to complete response may actually be shorter than that which was recorded. At the time of data cut-off, the median duration of complete response was greater than 8 months and ranged to 25+ months. Among 93 responding patients, seven had shown evidence of disease progression at the time of the data cut-off. In four of these patients, disease was limited to the bone marrow without peripheral blood abnormalities (pathologic progression), while in three patients there were also peripheral blood abnormalities (clinical progression). Seven patients who did not respond to a first course of LEUSTATIN received a second course of therapy. In the five patients who had adequate follow-up, additional courses did not appear to improve their overall response.

INDICATIONS FOR USE

LEUSTATIN Injection is indicated for the treatment of active Hairy Cell Leukemia as defined by clinically significant anemia, neutropenia, thrombocytopenia or disease-related symptoms.

CONTRAINDICATIONS

LEUSTATIN Injection is contraindicated in those patients who are hypersensitive to this drug or any of its components.

WARNINGS

Due to increased risk of infection in the setting of immunosuppression with chemotherapy including LEUSTATIN, it is recommended not to administer live attenuated vaccines to patients receiving LEUSTATIN Injection.

Severe bone marrow suppression, including neutropenia, anemia and thrombocytopenia, has been commonly observed in patients treated with LEUSTATIN, especially at high doses. At initiation of treatment, most patients in the clinical studies had hematologic impairment as a manifestation of active Hairy Cell

Leukemia. Following treatment with LEUSTATIN, further hematologic impairment occurred before recovery of peripheral blood counts began. During the first two weeks after treatment initiation, mean Platelet Count, ANC, and Hemoglobin concentration declined and subsequently increased with normalization of mean counts by Day 12, Week 5 and Week 8, respectively. The myelosuppressive effects of LEUSTATIN were most notable during the first month following treatment. Forty-four percent (44%) of patients received transfusions with RBCs and 14% received transfusions with platelets during Month 1. Careful hematologic monitoring, especially during the first 4 to 8 weeks after treatment with LEUSTATIN Injection, is recommended (see PRECAUTIONS).

Fever ($T \geq 100°F$) was associated with the use of LEUSTATIN in approximately two-thirds of patients (131/196) in the first month of therapy. Virtually all of these patients were treated empirically with parenteral antibiotics. Overall, 47% (93/196) of all patients had fever in the setting of neutropenia ($ANC \leq 1000$), including 62 patients (32%) with severe neutropenia (i.e., $ANC \leq 500$).

In a Phase I investigational study using LEUSTATIN in high doses (4 to 9 times the recommended dose for Hairy Cell Leukemia) as part of a bone marrow transplant conditioning regimen, which also included high dose cyclophosphamide and total body irradiation, acute nephrotoxicity and delayed onset neurotoxicity were observed. Thirty-one (31) poor-risk patients with drug-resistant acute leukemia in relapse (29 cases) or non-Hodgkins Lymphoma (2 cases) received LEUSTATIN for 7 to 14 days prior to bone marrow transplantation. During infusion, 8 patients experienced gastrointestinal symptoms. While the bone marrow was initially cleared of all hematopoietic elements, including tumor cells, leukemia eventually recurred in all treated patients. Within 7 to 13 days after starting treatment with LEUSTATIN, 6 patients (19%) developed manifestations of renal dysfunction (e.g., acidosis, anuria, elevated serum creatinine, etc.) and 5 required dialysis. Several of these patients were also being treated with other medications having known nephrotoxic potential. Renal dysfunction was reversible in 2 of these patients. In the 4 patients whose renal function had not recovered at the time of death, autopsies were performed; in 2 of these, evidence of tubular damage was noted. Eleven (11) patients (35%) experienced delayed onset neurologic toxicity. In the majority, this was characterized by progressive irreversible motor weakness (paraparesis/quadriparesis) of the upper and/or lower extremities, first noted 35 to 84 days after starting high dose therapy with LEUSTATIN. Non-invasive testing (electromyography and nerve conduction studies) was consistent with demyelinating disease. Severe neurologic toxicity has also been noted with high doses of another drug in this class.

Axonal peripheral polyneuropathy was observed in a dose escalation study at the highest dose levels (approximately 4 times the recommended dose for Hairy Cell Leukemia) in patients not receiving cyclophosphamide or total body irradiation. Severe neurological toxicity has been reported rarely following treatment with standard cladribine dosing regimens.

In patients with Hairy Cell Leukemia treated with the recommended treatment regimen (0.09 mg/kg/day for 7 consecutive days), there have been no reports of nephrologic toxicities.

Serious (e.g. respiratory infection, pneumonia and viral skin infections), including fatal infections (e.g. sepsis) were reported (see ADVERSE REACTIONS).

Of the 196 Hairy Cell Leukemia patients entered in the two trials, there were 8 deaths following treatment. Of these, 6 were of infectious etiology, including 3 pneumonias, and 2 occurred in the first month following LEUSTATIN therapy. Of the 8 deaths, 6 occurred in previously treated patients who were refractory to α interferon.

Benzyl alcohol is a constituent of the recommended diluent for the 7-day infusion solution. Benzyl alcohol has been reported to be associated with a fatal "Gasping Syndrome" in premature infants. (see DOSAGE AND ADMINISTRATION)

Pregnancy Category D:

LEUSTATIN can cause fetal harm when administered to a pregnant woman. Although there is no evidence of teratogenicity in humans due to LEUSTATIN, other drugs which inhibit DNA synthesis have been reported to be teratogenic in humans. Cladribine is teratogenic in animals. Advise females of reproductive potential to use highly effective contraception during treatment with LEUSTATIN. If LEUSTATIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

Cladribine is teratogenic in mice and rabbits and consequently has the potential to cause fetal harm when administered to a pregnant woman. A significant increase in fetal variations was observed in mice receiving 1.5 mg/kg/day (4.5 mg/m^2) and increased resorptions, reduced litter size and increased fetal malformations were observed when mice received 3.0 mg/kg/day (9 mg/m^2). Fetal death and malformations were observed in rabbits that received 3.0 mg/kg/day (33.0 mg/m^2). No fetal effects were seen in mice at 0.5 mg/kg/day (1.5 mg/m^2) or in rabbits at 1.0 mg/kg/day (11.0 mg/m^2).

PRECAUTIONS

General:

LEUSTATIN Injection is a potent antineoplastic agent with potentially significant toxic side effects. It should be administered only under the supervision of a physician experienced with the use of cancer chemotherapeutic agents. Patients undergoing therapy should be closely observed for signs of hematologic and non-hematologic toxicity. Periodic assessment of peripheral blood counts, particularly during the first 4 to 8 weeks post-treatment, is recommended to detect the development of anemia, neutropenia and thrombocytopenia and for early detection of any potential sequelae (e.g., infection or bleeding). As with other potent chemotherapeutic agents, monitoring of renal and hepatic function is also recommended, especially in patients with underlying kidney or liver dysfunction (see WARNINGS and ADVERSE REACTIONS).

Fever was a frequently observed side effect during the first month on study. Since the majority of fevers occurred in neutropenic patients, patients should be closely monitored during the first month of treatment and empiric antibiotics should be initiated as clinically indicated. Although 69% of patients developed fevers, less than 1/3 of febrile events were associated with documented infection. Given the known myelosuppressive effects of LEUSTATIN, practitioners should carefully evaluate the risks and benefits of administering this drug to patients with active infections (see WARNINGS and ADVERSE REACTIONS).

There are inadequate data on dosing of patients with renal or hepatic insufficiency. Development of acute renal insufficiency in some patients receiving high doses of LEUSTATIN has been described. Until more information is available, caution is advised when administering the drug to patients with known or suspected renal or hepatic insufficiency (see WARNINGS).

Rare cases of tumor lysis syndrome have been reported in patients treated with cladribine with other hematologic malignancies having a high tumor burden.

LEUSTATIN Injection must be diluted in designated intravenous solutions prior to administration (see DOSAGE AND ADMINISTRATION).

Laboratory Tests:

During and following treatment, the patient's hematologic profile should be monitored regularly to determine the degree of hematopoietic suppression. In the clinical studies, following reversible declines in all cell counts, the mean Platelet Count reached 100 x 10^9/L by Day 12, the mean Absolute Neutrophil Count reached 1500 x 10^6/L by Week 5 and the mean Hemoglobin reached 12 g/dL by Week 8.

After peripheral counts have normalized, bone marrow aspiration and biopsy should be performed to confirm response to treatment with LEUSTATIN. Febrile events should be investigated with appropriate laboratory and radiologic studies. Periodic assessment of renal function and hepatic function should be performed as clinically indicated.

Drug Interactions:

There are no known drug interactions with LEUSTATIN Injection. Caution should be exercised if LEUSTATIN Injection is administered before, after, or in conjunction with other drugs known to cause immunosuppression or myelosuppression. (see WARNINGS)

Carcinogenesis:

No animal carcinogenicity studies have been conducted with cladribine. However, its carcinogenic potential cannot be excluded based on demonstrated genotoxicity of cladribine.

Mutagenesis:

As expected for compounds in this class, the actions of cladribine yield DNA damage. In mammalian cells in culture, cladribine caused the accumulation of DNA strand breaks. Cladribine was also incorporated into DNA of human lymphoblastic leukemia cells. Cladribine was not mutagenic *in vitro* (Ames and Chinese hamster ovary cell gene mutation tests) and did not induce unscheduled DNA synthesis in primary rat hepatocyte cultures. However, cladribine was clastogenic both *in vitro* (chromosome aberrations in Chinese hamster ovary cells) and *in vivo* (mouse bone marrow micronucleus test).

Impairment of Fertility:

The effect on human fertility is unknown. When administered intravenously to Cynomolgus monkeys, cladribine has been shown to cause suppression of rapidly generating cells, including testicular cells.

Pregnancy:

Pregnancy Category D: (see WARNINGS).

Nursing Mothers:

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from cladribine, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug for the mother.

Pediatric Use:

Safety and effectiveness in pediatric patients have not been established. In a Phase I study involving patients 1-21 years old with relapsed acute leukemia, LEUSTATIN was given by continuous intravenous infusion in doses ranging from 3 to 10.7 mg/m^2/day for 5 days (one-half to twice the dose recommended in Hairy Cell Leukemia). In this study, the dose-limiting toxicity was severe myelosuppression with profound neutropenia and thrombocytopenia. At the highest dose (10.7 mg/m^2/day), 3 of 7 patients developed irreversible myelosuppression and fatal systemic bacterial or fungal infections. No unique toxicities were noted in this study [(1)] (see WARNINGS and ADVERSE REACTIONS).

Geriatric Use

Clinical studies of LEUSTATIN did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients. In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy in elderly patients.

ADVERSE REACTIONS

Clinical Trials Experience

Adverse drug reactions reported by ≥ 1% of LEUSTATIN-treated patients with HCL noted in the HCL clinical dataset (studies K90-091 and L91-048, n=576) are shown in the table below.

Adverse Drug Reactions in ≥ 1% of Patients Treated With LEUSTATIN in HCL Clinical Trials	
System Organ Class Preferred Term	**LEUSTATIN (n=576)** **%**
Blood and Lymphatic System Disorder (see also sections WARNINGS and PRECAUTIONS)	
Anemia	1
Febrile neutropenia	8
Psychiatric Disorders	
Anxiety	1
Insomnia	3
Nervous System Disorders	
Dizziness	6
Headache	14
Cardiac Disorders	
Tachycardia	2
Respiratory, Thoracic and Mediastinal Disorders	
Breath sounds abnormal	4
Cough	7
Dyspnea*	5
Rales	1
Gastrointestinal Disorders	
Abdominal pain**	4

Constipation	4
Diarrhea	7
Flatulence	1
Nausea	22
Vomiting	9
Skin and Subcutaneous Tissue Disorders	
Ecchymosis	2
Hyperhidrosis	3
Petechiae	2
Pruritus	2
Rash***	16
Musculoskeletal, Connective Tissue, and Bone Disorders	
Arthralgia	3
Myalgia	6
Pain****	6
General Disorders and Administration Site Conditions (see also sections WARNINGS and PRECAUTIONS)	
Administration site reaction*****	11
Asthenia	6
Chills	2
Decreased appetite	8
Fatigue	31
Malaise	5
Muscular weakness	1
Edema peripheral	2
Pyrexia	33
Injury, Poisoning and Procedural Complications	
Contusion	1

* Dyspnea includes dyspnea, dyspnea exertional, and wheezing
** Abdominal pain includes abdominal discomfort, abdominal pain, and abdominal pain (lower and upper)
*** Rash includes erythema, rash, and rash (macular, macula-papular, papular, pruritic, pustular and erythematous)
**** Pain includes pain, back pain, chest pain, arthritis pain, bone pain, and pain in extremity
***** Administration site reaction includes administration site reaction, catheter site (cellulitis, erythema, hemorrhage, and pain), and infusion site reaction(erythema, edema, and pain)

The following safety data are based on 196 patients with Hairy Cell Leukemia: the original cohort of 124 patients plus an additional 72 patients enrolled at the same two centers after the original enrollment cutoff. In Month 1 of the Hairy Cell Leukemia clinical trials, severe neutropenia was noted in 70% of patients, fever in 69%, and infection was documented in 28%. Most non-hematologic adverse experiences were mild to moderate in severity.

Myelosuppression was frequently observed during the first month after starting treatment. Neutropenia (ANC < 500 x 10^6/L) was noted in 70% of patients, compared with 26% in whom it was present initially. Severe anemia (Hemoglobin < 8.5 g/dL) developed in 37% of patients, compared with 10% initially and thrombocytopenia (Platelets < 20 x 10^9/L) developed in 12% of patients, compared to 4% in whom it was noted initially.

During the first month, 54 of 196 patients (28%) exhibited documented evidence of infection. Serious infections (e.g., septicemia, pneumonia) were reported in 6% of all

patients; the remainder were mild or moderate. Several deaths were attributable to infection and/or complications related to the underlying disease. During the second month, the overall rate of documented infection was 6%; these infections were mild to moderate and no severe systemic infections were seen. After the third month, the monthly incidence of infection was either less than or equal to that of the months immediately preceding LEUSTATIN therapy.

During the first month, 11% of patients experienced severe fever (i.e., ≥104°F). Documented infections were noted in fewer than one-third of febrile episodes. Of the 196 patients studied, 19 were noted to have a documented infection in the month prior to treatment. In the month following treatment, there were 54 episodes of documented infection: 23 (42%) were bacterial, 11 (20%) were viral and 11 (20%) were fungal. Seven (7) of 8 documented episodes of herpes zoster occurred during the month following treatment. Fourteen (14) of 16 episodes of documented fungal infections occurred in the first two months following treatment. Virtually all of these patients were treated empirically with antibiotics. (see WARNINGS and PRECAUTIONS)

Analysis of lymphocyte subsets indicates that treatment with cladribine is associated with prolonged depression of the CD4 counts. Prior to treatment, the mean CD4 count was 766/μL. The mean CD4 count nadir, which occurred 4 to 6 months following treatment, was 272/μL. Fifteen (15) months after treatment, mean CD4 counts remained below 500/μL. CD8 counts behaved similarly, though increasing counts were observed after 9 months. The clinical significance of the prolonged CD4 lymphopenia is unclear.

Another event of unknown clinical significance includes the observation of prolonged bone marrow hypocellularity. Bone marrow cellularity of < 35% was noted after 4 months in 42 of 124 patients (34%) treated in the two pivotal trials. This hypocellularity was noted as late as day 1010. It is not known whether the hypocellularity is the result of disease related marrow fibrosis or if it is the result of cladribine toxicity. There was no apparent clinical effect on the peripheral blood counts.

The vast majority of rashes were mild. Most episodes of nausea were mild, not accompanied by vomiting, and did not require treatment with antiemetics. In patients requiring antiemetics, nausea was easily controlled, most frequently with chlorpromazine.

When used in other clinical settings the following ADRs were reported: bacteremia, cellulitis, localized infection, pneumonia, anemia, thrombocytopenia (with bleeding or petechiae), phlebitis, purpura, crepitations, localized edema and edema.

For a description of adverse reactions associated with use of high doses in non-Hairy Cell Leukemia patients, see WARNINGS.

Postmarketing Experience

The following additional adverse reactions have been reported since the drug became commercially available. These adverse reactions have been reported primarily in patients who received multiple courses of LEUSTATIN Injection:

Infections and infestations: Septic shock. Opportunistic infections have occurred in the acute phase of treatment.

Blood and lymphatic system disorders: Bone marrow suppression with prolonged pancytopenia, including some reports of aplastic anemia; hemolytic anemia (including autoimmune hemolytic anemia), which was reported in patients with lymphoid malignancies, occurring within the first few weeks following treatment. Rare cases of myelodysplastic syndrome have been reported.

Immune system disorders: Hypersensitivity.

Metabolism and nutrition disorders: Tumor lysis syndrome.

Psychiatric disorders: Confusion (including disorientation).

Hepatobiliary disorders: Reversible, generally mild increases in bilirubin (uncommon) and transaminases.

Nervous System disorders: Depressed level of consciousness, neurological toxicity (including peripheral sensory neuropathy, motor neuropathy (paralysis), polyneuropathy, paraparesis); however, severe neurotoxicity has been reported rarely following treatment with standard cladribine dosing regimens.

Eye disorders: Conjunctivitis.

Respiratory, thoracic and mediastinal disorders: Pulmonary interstitial infiltrates (including lung infiltration, interstitial lung disease, pneumonitis and pulmonary fibrosis); in most cases, an infectious etiology was identified.

Skin and tissue disorders: Urticaria, hypereosinophilia; Stevens-Johnson. In isolated cases toxic epidermal necrolysis has been reported in patients who were receiving or had recently been treated with other medications (e.g., allopurinol or antibiotics) known to cause these syndromes.

Renal and urinary disorders: Renal failure (including renal failure acute, renal impairment).

OVERDOSAGE

High doses of LEUSTATIN have been associated with: irreversible neurologic toxicity (paraparesis/quadriparesis), acute nephrotoxicity, and severe bone marrow suppression resulting in neutropenia, anemia and thrombocytopenia (see WARNINGS). There is no known specific antidote to overdosage. Treatment of overdosage consists of discontinuation of LEUSTATIN, careful observation and appropriate supportive measures. It is not known whether the drug can be removed from the circulation by dialysis or hemofiltration.

DOSAGE AND ADMINISTRATION

Usual Dose:

The recommended dose and schedule of LEUSTATIN Injection for active Hairy Cell Leukemia is as a single course given by continuous infusion for 7 consecutive days at a dose of 0.09 mg/kg/day. Deviations from this dosage regimen are not advised. If the patient does not respond to the initial course of LEUSTATIN Injection for Hairy Cell Leukemia, it is unlikely that they will benefit from additional courses. Physicians should consider delaying or discontinuing the drug if neurotoxicity or renal toxicity occurs (see WARNINGS).

Specific risk factors predisposing to increased toxicity from LEUSTATIN have not been defined. In view of the known toxicities of agents of this class, it would be prudent to proceed carefully in patients with known or suspected renal insufficiency or severe bone marrow impairment of any etiology. Patients should be monitored closely for hematologic and non-hematologic toxicity (see WARNINGS and PRECAUTIONS).

Preparation and Administration of Intravenous Solutions:

LEUSTATIN Injection must be diluted with the designated diluent prior to administration. Since the drug product does not contain any anti-microbial preservative or bacteriostatic agent, **aseptic technique and proper environmental precautions must be observed in preparation of LEUSTATIN Injection solutions.**

To prepare a single daily dose:

LEUSTATIN Injection should be passed through a sterile 0.22µm disposable hydrophilic syringe filter prior to introduction into the infusion bag, prior to each daily infusion. Add the calculated dose (0.09 mg/kg or 0.09 mL/kg) of LEUSTATIN Injection through the sterile filter to an infusion bag containing 500 mL of 0.9% Sodium Chloride Injection, USP. Infuse continuously over 24 hours. Repeat daily for a total of 7 consecutive days. **The use of 5% dextrose as a diluent is not recommended because of increased degradation of cladribine.** Admixtures of

LEUSTATIN Injection are chemically and physically stable for at least 24 hours at room temperature under normal room fluorescent light in Baxter Viaflex®† PVC infusion containers. **Since limited compatibility data are available, adherence to the recommended diluents and infusion systems is advised.**

	Dose of LEUSTATIN Injection	Recommended Diluent	Quantity of Diluent
24-hour infusion method	1(day) x 0.09 mg/kg	0.9% Sodium Chloride Injection, USP	500 mL

To prepare a 7-day infusion:

The 7-day infusion solution should only be prepared with Bacteriostatic 0.9% Sodium Chloride Injection, USP (0.9% benzyl alcohol preserved). In order to minimize the risk of microbial contamination, both LEUSTATIN Injection and the diluent should be passed through a sterile 0.22 µm disposable hydrophilic syringe filter as each solution is being introduced into the infusion reservoir. First add the calculated dose of LEUSTATIN Injection (7 days x 0.09 mg/kg or mL/kg) to the infusion reservoir through the sterile filter.

Then add a calculated amount of Bacteriostatic 0.9% Sodium Chloride Injection, USP (0.9% benzyl alcohol preserved) also through the filter to bring the total volume of the solution to 100 mL. After completing solution preparation, clamp off the line, disconnect and discard the filter. Aseptically aspirate air bubbles from the reservoir as necessary using the syringe and a dry second sterile filter or a sterile vent filter assembly. Reclamp the line and discard the syringe and filter assembly. Infuse continuously over 7 days. Solutions prepared with Bacteriostatic Sodium Chloride Injection for individuals weighing more than 85 kg may have reduced preservative effectiveness due to greater dilution of the benzyl alcohol preservative. Admixtures for the 7-day infusion have demonstrated acceptable chemical and physical stability for at least 7 days in the SIMS Deltec MEDICATION CASSETTE™ Reservoir‡.

	Dose of LEUSTATIN Injection	Recommended Diluent	Quantity of Diluent
7-day infusion method (use sterile 0.22µ filter when preparing infusion solution)	7 (days) x 0.09 mg/kg	Bacteriostatic 0.9% Sodium Chloride Injection, USP (0.9% benzyl alcohol)	q.s. to 100 mL

Since limited compatibility data are available, adherence to the recommended diluents and infusion systems is advised. Solutions containing LEUSTATIN Injection should not be mixed with other intravenous drugs or additives or infused

simultaneously via a common intravenous line, since compatibility testing has not been performed. Preparations containing benzyl alcohol should not be used in neonates (see WARNINGS).

Care must be taken to assure the sterility of prepared solutions. Once diluted, solutions of LEUSTATIN Injection should be administered promptly or stored in the refrigerator (2° to 8° C) for no more than 8 hours prior to start of administration. Vials of LEUSTATIN Injection are for single-use only. Any unused portion should be discarded in an appropriate manner (see Handling and Disposal).

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. A precipitate may occur during the exposure of LEUSTATIN Injection to low temperatures; it may be resolubilized by allowing the solution to warm naturally to room temperature and by shaking vigorously. **DO NOT HEAT OR MICROWAVE.**

Chemical Stability of Vials:

When stored in refrigerated conditions between 2° to 8°C (36° to 46°F) protected from light, unopened vials of LEUSTATIN Injection are stable until the expiration date indicated on the package. Freezing does not adversely affect the solution. If freezing occurs, thaw naturally to room temperature. DO NOT heat or microwave. Once thawed, the vial of LEUSTATIN Injection is stable until expiry if refrigerated. DO NOT refreeze. Once diluted, solutions containing LEUSTATIN Injection should be administered promptly or stored in the refrigerator (2° to 8°C) for no more than 8 hours prior to administration.

Handling and Disposal:

The potential hazards associated with cytotoxic agents are well established and proper precautions should be taken when handling, preparing, and administering LEUSTATIN Injection. The use of disposable gloves and protective garments is recommended. If LEUSTATIN Injection contacts the skin or mucous membranes, wash the involved surface immediately with copious amounts of water. Several guidelines on this subject have been published.[2-8] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate. Refer to your Institution's guidelines and all applicable state/local regulations for disposal of cytotoxic waste.

HOW SUPPLIED

LEUSTATIN Injection is supplied as a sterile, preservative-free, isotonic solution containing 10 mg (1 mg/mL) of cladribine as 10 mL filled into a single-use clear flint

glass 20 mL vial. LEUSTATIN Injection is supplied in 10 mL (1 mg/mL) single-use vials (NDC 59676-201-01) available in a treatment set (case) of seven vials.

Store refrigerated 2° to 8°C (36° to 46°F). Protect from light during storage.

REFERENCES:

1. Santana VM, Mirro J, Harwood FC, *et al*: A phase I clinical trial of 2-Chloro-deoxyadenosine in pediatric patients with acute leukemia. J. Clin. Onc., **9**: 416 (1991).

2. Recommendations for the Safe Handling of Parenteral Antineoplastic Drugs. NIH Publication No. 83-2621. For sale by the Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 20402.

3. AMA Council Report. Guidelines for Handling Parenteral Antineoplastics, JAMA, March 15 (1985).

4. National Study Commission on Cytotoxic Exposure--Recommendations for Handling Cytotoxic Agents. Available from Louis P. Jeffrey, Sc.D., Chairman, National Study Commission on Cytotoxic Exposure, Massachusetts College of Pharmacy and Allied Health Sciences, 179 Longwood Avenue, Boston, Massachusetts 02115.

5. Clinical Oncological Society of Australia: Guidelines and Recommendations for Safe Handling of Antineoplastic Agents, Med. J. Australia **1**:425 (1983).

6. Jones RB, *et al.* Safe Handling of Chemotherapeutic Agents: A Report from the Mount Sinai Medical Center. Ca--A Cancer Journal for Clinicians, Sept/Oct. 258-263 (1983).

7. American Society of Hospital Pharmacists Technical Assistance Bulletin on Handling Cytotoxic Drugs in Hospitals. Am. J. Hosp. Pharm., **42**:131 (1985).

8. OSHA Work-Practice Guidelines for Personnel Dealing with Cytotoxic (antineoplastic) Drugs. Am. J. Hosp. Pharm., **43**:1193 (1986).

† Viaflex® containers, manufactured by Baxter Healthcare Corporation - Code No. 2B8013 (tested in 1991)

‡ MEDICATION CASSETTE™ Reservoir, manufactured by SIMS Deltec, Inc. - Reorder No. 602100A (tested in 1991)

Centocor Ortho Biotech Products, L.P.
Raritan, NJ 08869

[new code]
Revised July 2012

EXHIBIT T

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

75405

DRAFT FINAL PRINTED LABELING

CLADRIBINE INJECTION

Rx ONLY

For Intravenous Infusion Only

WARNINGS

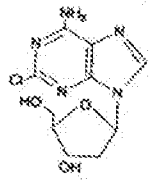
Cladribine injection should be administered under the supervision of a qualified physician experienced in the use of antineoplastic therapy. Suppression of bone marrow function should be anticipated. This is usually reversible and appears to be dose dependent. Serious neurological toxicity (including irreversible paraparesis and quadriparesis) has been reported in patients who received cladribine injection by continuous infusion at high doses (4 to 9 times the recommended dose for Hairy Cell Leukemia). Neurologic toxicity appears to demonstrate a dose relationship; however, severe neurological toxicity has been reported rarely following treatment with standard cladribine dosing regimens.

Acute nephrotoxicity has been observed with high doses of cladribine (4 to 9 times the recommended dose for Hairy Cell Leukemia), especially when given concomitantly with other nephrotoxic agents/therapies.

DESCRIPTION

Cladribine injection (also commonly known as 2-chloro-2'-deoxy-β-D-adenosine) is a synthetic antineoplastic agent for continuous intravenous infusion. It is a clear, colorless, sterile, preservative-free, isotonic solution. Cladribine injection is available in single-use vials containing 10 mg (1 mg/mL) of cladribine, a chlorinated purine nucleoside analog. Each mL of cladribine injection contains 1 mg of the active ingredient and 9 mg (0.15 mEq) of sodium chloride as an inactive ingredient. The solution has a pH range of 5.5 to 8.0. Phosphoric acid and/or dibasic sodium phosphate may have been added to adjust the pH to 6.3 ± 0.3.

The chemical name for cladribine is 2-chloro-6-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine and the structure is represented below:



Molecular Formula = $C_{10}H_{12}ClN_5O_3$ Molecular Weight = 285.69

CLINICAL PHARMACOLOGY

Cellular Resistance and Sensitivity:

The selective toxicity of 2-chloro-2'-deoxy-β-D-adenosine towards certain normal and malignant lymphocyte and monocyte populations is based on the relative activities of deoxycytidine kinase and deoxynucleotidase. Cladribine passively crosses the cell membrane. In cells with a high ratio of deoxycytidine kinase to deoxynucleotidase, it is phosphorylated by deoxycytidine kinase to 2-chloro-2'-deoxy-β-D-adenosine monophosphate (2-CdAMP). Since 2-chloro-2'-deoxy-β-D-adenosine is resistant to deamination by adenosine deaminase and there is little deoxynucleotide deaminase in lymphocytes and monocytes, 2-CdAMP accumulates intracellularly and is subsequently converted into the active triphosphate deoxynucleotide, 2-chloro-2'-deoxy-β-D-adenosine triphosphate (2-CdATP). It is postulated that cells with high deoxycytidine kinase and low deoxynucleotidase activities will be selectively killed by 2-chloro-2'-deoxy-β-D-adenosine as toxic deoxynucleotides accumulate intracellularly.

Cells containing high concentrations of deoxynucleotides are unable to properly repair single-strand DNA breaks. The broken ends of DNA activate the enzyme poly (ADP-ribose) polymerase resulting in NAD and ATP depletion and disruption of cellular metabolism. There is evidence, also, that 2-CdATP is incorporated into the DNA of dividing cells, resulting in impairment of DNA synthesis. Thus, 2-chloro-2'-deoxy-β-D-adenosine can be distinguished from other chemotherapeutic agents affecting purine metabolism in that it is cytotoxic to both actively dividing and quiescent lymphocytes and monocytes, inhibiting both DNA synthesis and repair.

HUMAN PHARMACOLOGY

In a clinical investigation, 17 patients with Hairy Cell Leukemia and normal renal function were treated for 7 days with the recommended treatment regimen of cladribine (0.09 mg/kg/day) by continuous intravenous infusion. The mean steady-state serum concentration was estimated to be 5.7 ng/mL with an estimated systemic clearance of 663.5 mL/h/kg when cladribine was given by continuous infusion over 7 days. In Hairy Cell Leukemia patients, there does not appear to be a relationship between serum concentrations and ultimate clinical outcome.

In another study, 8 patients with hematologic malignancies received a two (2) hour infusion of cladribine (0.12 mg/kg). The mean end-of-infusion plasma cladribine concentration was 48 ± 19 ng/mL. For 5 of these patients, the disappearance of cladribine could be described by either a biphasic or triphasic decline. For these patients with normal renal function, the mean terminal half-life was 5.4 hours. Mean values for clearance and steady-state volume of distribution were 978 ± 422 mL/h/kg and 8.5 ± 2.8 L/kg, respectively.

Plasma concentrations are reported to decline multi-exponentially after intravenous infusions with terminal half-lives ranging from approximately 3 to 22 hours. In general, the apparent volume of distribution of cladribine is very large (mean approximately 9 L/kg), indicating an extensive distribution of cladribine in body tissues. The mean half-life of cladribine in leukemic cells has been reported to be 23 hours.

Cladribine penetrates into cerebrospinal fluid. One report indicates that concentrations are approximately 20% of those in plasma.

Cladribine is bound approximately 20% to plasma proteins.

Except for some understanding of the mechanism of cellular toxicity, no other information is available on the metabolism of cladribine in humans. An average of 18% of the administered dose has been reported to be excreted in urine of patients with solid tumors during a 5-day continuous intravenous infusion of 3.5 to 8.1 mg/m²/day of cladribine. The effect of renal and hepatic impairment on the elimination of cladribine has not been investigated in humans.

Two single-center open label studies of cladribine have been conducted in patients with Hairy Cell Leukemia with evidence of active disease requiring therapy. In the study conducted at the Scripps Clinic and Research Foundation (Study A), 68 patients were treated with a single course of cladribine given by continuous intravenous infusion for 7 days at a dose of 0.09 mg/kg/day. In the study conducted at the M.D. Anderson Cancer Center (Study B), 35 patients were treated with a 7 day continuous intravenous infusion of cladribine at a comparable dose of 3.5 mg/m²/day. A complete response (CR) required clearing of the peripheral blood and bone marrow of hairy cells and recovery of the hemoglobin to 12 g/dL, platelet count to 100 x 10⁹/L, and absolute neutrophil count to 1500 x 10⁹/L. A good partial response (GPR) required the same hematologic parameters as a complete response, and that fewer than 5% hairy cells remain in the bone marrow. A partial response (PR) required that hairy cells in the bone marrow be decreased by at least 50% from baseline and the same response for hematologic parameters as for complete response. A pathologic relapse was defined as an increase in bone marrow hairy cells to 25% of pretreatment levels. A clinical relapse was defined as the recurrence of cytopenias, specifically, decreases in hemoglobin ≥ 2 g/dL, ANC ≤ 25% or platelet counts ≤ 50,000. Patients who met the criteria for a complete response but subsequently were found to have evidence of bone marrow hairy cells (>25% of pretreatment levels) were reclassified as partial responses and were not considered to be complete responses with relapse.

Among patients evaluable for efficacy (N=106), using the hematologic and bone marrow response criteria described above, the complete response rates in patients treated with cladribine were 65% and 68% for Study A and Study B, respectively, yielding a combined complete response rate of 66%. Overall response rates (i.e., Complete plus Good Partial plus Partial Responses) were 89% and 80% in Study A and Study B, respectively, for a combined overall response rate of 88% in evaluable patients treated with cladribine.

Using an intent-to-treat analysis (N=123) and further requiring no evidence of splenomegaly as a criterion for CR (i.e., no palpable spleen on physical examination and ≤ 13 cm on CT scan), the complete response rates for Study A and Study B were 54% and 52%, respectively, giving a combined CR rate of 54%. The overall response rates (CR + GPR + PR) were 90% and 85%, for Studies A and B, respectively, yielding a combined overall response rate of 89%.

RESPONSE RATES TO CLADRIBINE TREATMENT IN PATIENTS WITH HAIRY CELL LEUKEMIA

	CR	Overall
Evaluable Patients N=106	66%	88%
Intent-to-Treat Population N=123	54%	89%

In these studies, 60% of the patients had not received prior chemotherapy for Hairy Cell Leukemia or had undergone splenectomy as the only prior treatment and were receiving cladribine as a first-line treatment. The remaining 40% of the patients received cladribine as a second-line treatment, having been treated previously with other agents, including α-interferon and/or deoxycoformycin. The overall response rate for patients without prior chemotherapy was 90%, compared with 84% for previously treated patients. Cladribine is active in previously treated patients; however, retrospective analysis suggests that the overall response rate is decreased in patients previously treated with splenectomy or deoxycoformycin and in patients refractory to α-interferon.

OVERALL RESPONSE RATES (CR + CP + PR) TO CLADRIBINE TREATMENT IN PATIENTS WITH HAIRY CELL LEUKEMIA

	OVERALL RESPONSE (N=123)	NR + RELAPSE
No Prior Chemotherapy	68/74	6 + 4
	92%	14%
Any Prior Chemotherapy	41/49	8 + 3
	84%	22%
Previous Splenectomy	32/41*	9 + 1
	78%	24%
Previous Interferon	40/48	8 + 3
	83%	23%
Interferon Refractory	6/11*	3 + 2
	55%	84%
Previous Deoxycoformycin	3/5*	2 + 1
	59%	68%

NR = No Response
*P<0.05

After reversible decline, normalization of peripheral blood counts (Hemoglobin >12 g/dL, Platelets >100 x 10⁹/L, Absolute Neutrophil Count (ANC) >1500 x 10⁶/L) was achieved by 82% of evaluable patients. The median time to normalization of peripheral counts was 8 weeks from the start of treatment (Range: 2 to 22). The median time to normalization of Platelet Count was 2 weeks, the median time to normalization of ANC was 5 weeks and the median time to normalization of Hemoglobin was 8 weeks. With normalization of Platelet Count and Hemoglobin, requirements for platelet and RBC transfusions were abolished after Months 1 and 2, respectively, in those patients with complete response. Platelet recovery may be delayed in a majority of patients with severe baseline thrombocytopenia. Corresponding to normalization of ANC, a trend toward a reduced incidence of infection was seen after the third month, when compared to the months immediately preceding cladribine therapy. See also **WARNINGS, PRECAUTIONS** and **ADVERSE REACTIONS**.

CLADRIBINE TREATMENT IN PATIENTS WITH HAIRY CELL LEUKEMIA
TIME TO NORMALIZATION OF PERIPHERAL BLOOD COUNTS

Parameter	Median Time to Normalization of Count*
Platelet Count	2 weeks
Absolute Neutrophil Count	5 weeks
Hemoglobin	8 weeks
ANC, Hemoglobin and Platelet Count	9 weeks

*Day 1 = First day of infusion

For patients achieving a complete response, the median time to response (i.e., absence of hairy cells in bone marrow and peripheral blood together with normalization of peripheral blood parameters), measured from treatment start, was approximately 4 months. Since bone marrow aspiration and biopsy were frequently not performed at the time of peripheral blood normalization, the median time to complete response may actually be shorter than that which was recorded. At the time of data cut-off, the median duration of complete response was greater than 8 months and ranged to 21+ months. Among 93 responding patients, seven had shown evidence of disease progression at the time of the data cut-off. In five of these patients, disease was limited to the bone marrow without peripheral blood abnormalities (pathologic progression), while in three patients there were also peripheral blood abnormalities (clinical progression). Seven patients who did not respond to a first course of cladribine received a second course of therapy. In the five patients who had adequate follow-up, additional courses did not appear to improve their overall response.

INDICATIONS AND USAGE

Cladribine is indicated for the treatment of active Hairy Cell Leukemia as defined by clinically significant anemia, neutropenia, thrombocytopenia or disease-related symptoms.

CONTRAINDICATIONS

Cladribine is contraindicated in those patients who are hypersensitive to this drug or any of its components.

WARNINGS

Severe bone marrow suppression, including neutropenia, anemia and thrombocytopenia, has been commonly observed in patients treated with cladribine, especially at high doses. At initiation of treatment, most patients in the clinical studies had hematologic impairment as a manifestation of active Hairy Cell Leukemia. Following treatment with cladribine, further hematologic impairment occurred before recovery of peripheral blood counts began. During the first two weeks after treatment initiation, mean Platelet Count, ANC, and Hemoglobin concentration declined and subsequently increased with normalization of mean counts by Day 12, Week 3 and Week 8, respectively. The myelosuppressive effects of cladribine were most notable during the first month following treatment. Forty-four percent (44%) of patients received transfusions with RBCs and 14% received transfusions with platelets during Month 1. Careful hematologic monitoring, especially during the first 4 to 8 weeks after treatment with cladribine, is recommended. See **PRECAUTIONS**.

Fever (T ≥ 100.8) was associated with the use of cladribine in approximately two-thirds of patients (131/196) in the first month of therapy. Virtually all of these patients were treated empirically with parenteral antibiotics. Overall, 47% (93/196) of all patients had fever in the setting of neutropenia (ANC ≤ 1000), including 62 patients (32%) with severe neutropenia (i.e., ANC ≤ 500).

In a Phase I investigational study using cladribine in high doses (4 to 6 times the recommended dose for Hairy Cell Leukemia) as part of a bone marrow transplant conditioning regimen, which also included high dose cyclophosphamide and total body irradiation, acute nephrotoxicity and delayed onset neurotoxicity were observed. Thirty-one (31) poor-risk patients with drug-resistant acute leukemia in relapse (29 cases) or non-Hodgkins Lymphoma (2 cases) received cladribine for 7 to 14 days prior to bone marrow transplantation. During infusion, 8 patients experienced gastrointestinal symptoms. While the bone marrow was initially cleared of all hematopoietic elements, including tumor cells, leukemia eventually recurred in all treated patients. Within 7 to 13 days after starting treatment with cladribine, 6 patients (19%) developed manifestations of renal dysfunction (e.g., acidosis, anuria, elevated serum creatinine, etc.) and 5 required dialysis. Several of these patients were also being treated with other medications having known nephrotoxic potential. Renal dysfunction was reversible in 2 of these patients. In the 4 patients whose renal function had not recovered at the time of death, autopsies were performed; in 2 of these, evidence of tubular damage was noted. Eleven (11) patients (35%) experienced delayed onset neurologic toxicity. In the majority, this was characterized by progressive sleepiness, weakness (paraparesis/quadriparesis), of the upper and/or lower extremities, first noted 33 to 84 days after starting high dose therapy with cladribine. Non-invasive testing (electromyography and nerve conduction studies) was consistent with demyelinating disease. Severe neurologic toxicity has also been noted with high doses of another drug in this class.

Axonal peripheral polyneuropathy was observed in a dose escalation study at the highest dose levels (approximately 4 times the recommended dose for Hairy Cell Leukemia) in patients not receiving cyclophosphamide or total body irradiation. Severe neurological toxicity has been reported rarely following treatment with standard cladribine dosing regimens.

In patients with Hairy Cell Leukemia treated with the recommended treatment regimen (0.09 mg/kg/day for 7 consecutive days), there have been no reports of neurologic toxicities.

Of the 196 Hairy Cell Leukemia patients entered in the two trials, there were 8 deaths following treatment. Of these, 6 were of infectious etiology, including 3 pneumonias, and 2 occurred in the first month following cladribine therapy. Of the 8 deaths, 6 occurred in previously treated patients who were Refractory to α-interferon.

Benzyl alcohol is a constituent of the recommended diluent for the 7-day infusion solution. Benzyl alcohol has been reported to be associated with a fatal "Gasping Syndrome" in premature infants. See **DOSAGE AND ADMINISTRATION**.

Pregnancy Category D: Cladribine should not be given during pregnancy.

Cladribine is teratogenic in mice and rabbits and consequently has the potential to cause fetal harm when administered to a pregnant woman. A significant increase in fetal variations was observed in mice receiving 1.5 mg/kg/day (4.5 mg/m²) and increased resorptions, reduced litter size and increased fetal malformations were observed when mice received 3 mg/kg/day (9 mg/m²). Fetal death and malformations were observed in rabbits that received 2 mg/kg/day

(33 mg/m²). No fetal effects were seen in mice at 0.5 mg/kg/day (3.5 mg/m²) or in rabbits at 1 mg/kg/day (11 mg/m²).

Although there is no evidence of teratogenicity in humans due to cladribine, other drugs which inhibit DNA synthesis (e.g., methotrexate and aminopterin) have been reported to be teratogenic in humans. Cladribine has been shown to be embryotoxic in mice when given at doses equivalent to the recommended dose.

There are no adequate and well controlled studies in pregnant women. If cladribine is used during pregnancy, or if the patient becomes pregnant while taking the drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing age should be advised to avoid becoming pregnant.

PRECAUTIONS

General: Cladribine is a potent antineoplastic agent with potentially significant toxic side effects. It should be administered only under the supervision of a physician experienced with the use of cancer chemotherapeutic agents. Patients undergoing therapy should be closely observed for signs of hematologic and non-hematologic toxicity. Periodic assessment of peripheral blood counts, particularly during the first 4 to 8 weeks post-treatment, is recommended to detect the development of anemia, neutropenia and thrombocytopenia and for early detection of any potential sequelae (e.g., infection or bleeding). As with other potent chemotherapeutic agents, monitoring of renal and hepatic function is also recommended, especially in patients with underlying kidney or liver dysfunction. See **WARNINGS** and **ADVERSE REACTIONS**.

Fever was a frequently observed side effect during the first month on study. Since the majority of fevers occurred in neutropenic patients, patients should be closely monitored during the first month of treatment and empiric antibiotics should be initiated as clinically indicated. Although 69% of patients developed fevers, less the 1/3 of febrile events were associated with documented infection.

Given the known myelosuppressive effects of cladribine, practitioners should carefully evaluate the risks and benefits of administering this drug to patients with active infections. See **WARNINGS** and **ADVERSE REACTIONS**.

There are inadequate data on dosing of patients with renal or hepatic insufficiency. Development of acute renal insufficiency in some patients receiving high doses of cladribine has been described. Until more information is available, caution is advised when administering the drug to patients with known or suspected renal or hepatic insufficiency. See **WARNINGS**.

Rare cases of tumor lysis syndrome have been reported in patients treated with cladribine with other hematologic malignancies having a high tumor burden.

Cladribine must be diluted in designated intravenous solutions prior to administration. See **DOSAGE AND ADMINISTRATION**.

Laboratory Tests: During and following treatment, the patient's hematologic profile should be monitored regularly to determine the degree of hematopoietic suppression. In the clinical studies, following reversible declines in all cell counts, the mean Platelet Count reached 100 x 10⁹/L by Day 12, the mean Absolute Neutrophil Count reached 1500 x 10⁶/L by Week 5 and the mean Hemoglobin reached 12 g/dL by Week 8. After peripheral counts have normalized, bone marrow aspiration and biopsy should be performed to confirm response to treatment with cladribine. Febrile events should be investigated with appropriate laboratory and radiologic studies. Periodic assessment of renal function and hepatic function should be performed as clinically indicated.

Drug Interactions: There are no known drug interactions with cladribine. Caution should be exercised if cladribine is administered before, after, or in conjunction with other drugs known to cause immunosuppression or myelosuppression. See **WARNINGS**.

Carcinogenesis, Mutagenesis, Impairment of Fertility: No animal carcinogenicity studies have been conducted with cladribine. However, its carcinogenic potential cannot be excluded based on demonstrated genotoxicity of cladribine.

As expected for compounds in this class, the actions of cladribine yield DNA damage. In mammalian cells in culture, cladribine caused the accumulation of DNA strand breaks. Cladribine was also incorporated into DNA of human lymphoblastic leukemia cells. Cladribine was not mutagenic *in vitro* (Ames and Chinese hamster ovary cell gene mutation tests) and did not induce unscheduled DNA synthesis in primary rat hepatocyte cultures. However, cladribine was clastogenic both *in vitro* (chromosome aberrations in Chinese hamster ovary cells) and *in vivo* (mouse bone marrow micronucleus test).

When administered intravenously to Cynomolgus monkeys, cladribine has been shown to cause suppression of rapidly generating cells, including testicular cells. The effect on human fertility is unknown.

Pregnancy: Teratogenic Effects: Pregnancy Category D. See **WARNINGS**.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from cladribine, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug for the mother.

Pediatric Use: Safety and effectiveness in pediatric patients have not been established. In a Phase I study involving patients 1 to 21 years old with relapsed acute leukemia, cladribine was given by continuous intravenous infusion in doses ranging from 3 to 10.7 mg/m²/day for 5 days (one-half to twice the dose recommended in Hairy Cell Leukemia). In this study, the dose-limiting toxicity was severe myelosuppression with profound neutropenia and thrombocytopenia. At the highest dose (10.7 mg/m²/day), 3 of 7 patients developed irreversible myelosuppression and fatal systemic bacterial or fungal infections. No unique toxicities were noted in this study. See **WARNINGS** and **ADVERSE REACTIONS**.

ADVERSE REACTIONS

Safety data are based on 196 patients with Hairy Cell Leukemia: the original cohort of 124 patients plus additional 72 patients enrolled at the same two centers after the original enrollment cutoff. In Month 1 of the Hairy Cell Leukemia clinical trials, severe neutropenia was noted in 70% of patients, fever in 69%, and infection was documented in 28%. Other adverse experiences reported frequently during the first 14 days after initiating treatment included: fatigue (45%), rashes (28%), rash (27%), headache (22%) and injection site reactions (19%). Most non-hematologic adverse experiences were mild to moderate in severity.

Myelosuppression was frequently observed during the first month after starting treatment. Neutropenia (ANC < 500 x 10⁶/L) was noted in 70% of patients, compared with 26% in whom it was present initially. Severe anemia (Hemoglobin <8.5 g/dL) developed in 37% of patients, compared with 10% initially and thrombocytopenia (Platelets < 20 x 10⁹/L) developed in 12% of patients, compared to 4% in whom it was noted initially.

During the first month, 54 of 196 patients (28%) exhibited documented evidence of infection. Serious infections (e.g., septicemia, pneumonia) were reported in 8% of all patients; the remainder were mild or moderate. Several deaths were attributable to infection and/or complications related to the underlying disease. During the second month, the overall rate of documented infection was 8%; these infections were mild to moderate and no severe systemic infections were seen. After the third month, the monthly incidence of infection was either less than or equal to that of the months immediately preceding cladribine therapy.

During the first month, 11% of patients experienced severe fever (i.e., ≥39.4°F). Documented infections were noted in fewer than one-third of febrile episodes. Of the 196 patients studied, 18 were noted to have a documented infection in the month prior to treatment. In the month following treatment, there were 54 episodes of documented infection: 23 (42%) were bacterial, 11 (20%) were viral and 11 (20%) were fungal. Seven (7) of 8 documented episodes of fungal sepsis occurred during the month following treatment. Fourteen (14) of 16 episodes of documented fungal infections occurred in the first two months following treatment. Virtually all of these patients were treated empirically with antibiotics. See **WARNINGS** and **PRECAUTIONS**.

Analysis of lymphocyte subsets indicates that treatment with cladribine is associated with prolonged depression of the CD4 counts. Prior to treatment, the mean CD4 count was 756/µL. The mean CD4 count nadir, which occurred 4 to 6 months following treatment, was 272/µL. Fifteen (15) months after treatment, mean CD4 counts remained below 500/µL. CD8 counts behaved similarly, though increasing counts were observed after 9 months. The clinical significance of the prolonged CD4 lymphopenia is unclear.

Another event of unknown clinical significance includes the observation of prolonged bone marrow hypocellularity. Bone marrow cellularity of <20% was noted after 4 months in 42 of 124 patients (34%) treated in the two pivotal trials. This hypocellularity was noted at late as day 1010. It is not known whether the hypocellularity is the result of disease related marrow fibrosis or if it is the result of cladribine toxicity. There was no apparent clinical effect on the peripheral blood counts.

The vast majority of rashes were mild and occurred in patients who were receiving or had recently been treated with other medications (e.g., allopurinol or antibiotics) known to cause rash.

Most episodes of nausea were mild, not accompanied by vomiting, and did not require treatment with antiemetics. In patients requiring antiemetics, nausea was easily controlled, most frequently with chlorpromazine.

Adverse reactions reported during the first 2 weeks following treatment initiation (regardless of relationship to drug) by >5% of patients included:

Body as a Whole: fever (69%), fatigue (45%), chills (9%), asthenia (9%), diaphoresis (9%), malaise (7%), trunk pain (6%)

Gastrointestinal: nausea (28%), decreased appetite (17%), vomiting (13%), diarrhea (10%), constipation (9%), abdominal pain (6%)

Hemic/Lymphatic: purpura (10%), petechiae (8%), epistaxis (3%)

Nervous System: headache (22%), dizziness (9%), insomnia (7%)

Cardiovascular System: edema (6%), tachycardia (6%)

Respiratory System: abnormal breath sounds (11%), cough (10%), abnormal chest sounds (9%), shortness of breath (7%)

Skin/Subcutaneous Tissue: rash (27%), injection site reactions (19%), pruritis (6%), pain (6%), erythema (6%)

Musculoskeletal System: myalgia (7%), arthralgia (5%)

Adverse experiences related to intravenous administration included: injection site reactions (9%) (i.e., redness, swelling, pain), thrombosis (2%), phlebitis (2%) and a broken catheter (1%).

These appear to be related to the infusion procedure and/or indwelling catheter, rather than the medication or the vehicle. From Day 15 to the last follow-up visit, the only events reported by >5% of patients were: fatigue (11%), rash (10%), headache (7%), cough (7%), and malaise (5%).

For a description of adverse reactions associated with use of high doses in non-Hairy Cell Leukemia patients, see **WARNINGS**.

The following additional adverse events have been reported since the drug became commercially available. These adverse events have been reported primarily in patients who received multiple courses of cladribine:

Hematologic: bone marrow suppression with prolonged pancytopenia, including some reports of aplastic anemia, hemolytic anemia, which was reported in patients with lymphoid malignancies, occurring within the first few weeks following treatment.

Hepatic: reversible, generally mild increases in bilirubin and transaminases.

Nervous System: Neurological toxicity, however, severe neurotoxicity has been reported rarely following treatment with standard cladribine dosing regimens.

Respiratory System: pulmonary interstitial infiltrates, in most cases, an infectious etiology was identified.

Skin/Subcutaneous: urticaria, hyperpigmentation. In isolated cases Stevens-Johnson and toxic epidermal necrolysis have been reported in patients who were receiving or had recently been treated with other medications (e.g., allopurinol or antibiotics) known to cause these syndromes.

Opportunistic infections have occurred in the acute phase of treatment due to the immunosuppression mediated by cladribine.

OVERDOSAGE

High doses of cladribine have been associated with: irreversible neurologic toxicity (paraparesis/quadriparesis), acute nephrotoxicity, and severe bone marrow suppression resulting in neutropenia, anemia and thrombocytopenia. See **WARNINGS**. There is no known specific antidote to overdosage. Treatment of overdosage consists of discontinuation of cladribine, careful observation and appropriate supportive measures. It is not known whether the drug can be removed from the circulation by dialysis or hemofiltration.

DOSAGE AND ADMINISTRATION

Usual Dose:

The recommended dose and schedule of cladribine for active Hairy Cell Leukemia is as a single course given by continuous infusion for 7 consecutive days at a dose of 0.09 mg/kg/day. Deviations from this dosage regimen are not advised. If the patient does not respond to the initial course of cladribine for Hairy Cell Leukemia, it is unlikely that they will benefit from additional courses. Physicians should consider delaying or discontinuing the drug if neurotoxicity or renal toxicity occurs. See **WARNINGS**.

Specific risk factors predisposing to increased toxicity from cladribine have not been defined. In view of the known toxicities of agents of this class, it would be prudent to proceed carefully in patients with known or suspected renal insufficiency or severe bone marrow impairment of any etiology. Patients should be monitored closely for hematologic and non-hematologic toxicity. See **WARNINGS** and **PRECAUTIONS**.

Preparation and Administration of Intravenous Solutions:

Cladribine must be diluted with the designated diluent prior to administration. Since the drug product does not contain any antimicrobial preservative or bacteriostatic agent, **aseptic technique and proper environmental precautions must be observed in preparation of cladribine solutions.**

To prepare a single daily dose: Add the calculated dose (0.09 mg/kg or 0.06 mL/kg) of cladribine to an infusion bag containing 500 mL of 0.9% Sodium Chloride Injection. Infuse continuously over 24 hours. Repeat daily for a total of 7 consecutive days. **The use of 5% dextrose as a diluent is not recommended because of increased degradation of cladribine.** Admixtures of cladribine are chemically and physically stable for at least 24 hours at room temperature under normal room fluorescent light in Baxter Viaflex® PVC infusion containers. **Since limited compatibility data are available, adherence to the recommended diluents and infusion systems is advised.**

	Dose of Cladribine Injection	Recommended Diluent	Quantity of Diluent
24 hour infusion method	1 (day) x 0.09 mg/kg	0.9% Sodium Chloride Injection	500 mL

To prepare a 7 day infusion: The 7 day infusion solution should only be prepared with Bacteriostatic 0.9% Sodium Chloride Injection (0.9% benzyl alcohol preserved). In order to minimize the risk of microbial contamination, both cladribine injection and the diluent should be passed through a sterile 0.22µ disposable hydrophilic syringe filter as each solution is being introduced into the infusion reservoir. First add the calculated dose of cladribine (7 days x 0.09 mg/kg or mL/kg) to the infusion reservoir through the sterile filter. Then add a calculated amount of Bacteriostatic 0.9% Sodium Chloride Injection (0.9% benzyl alcohol preserved) also through the filter to bring the total volume of the solution to 100 mL. After completing solution preparation, clamp off the line, disconnect and discard the filter. Aseptically aspirate air bubbles from the reservoir as necessary using the syringe and a dry second sterile filter or a sterile vent filter assembly. Reclamp the line and discard the syringe and filter assembly. Infuse continuously over 7 days. Solutions prepared with Bacteriostatic Sodium Chloride Injection for individuals weighing more than 85 kg may have reduced preservative effectiveness due to greater dilution of the benzyl alcohol preservative. Admixtures for the 7 day infusion have demonstrated acceptable chemical and physical stability for at least 7 days in the SIMS Deltec MEDICATION CASSETTE™ Reservoir 1.

	Dose of Cladribine Injection	Recommended Diluent	Quantity of Diluent
7 day infusion method (use sterile 0.22µ filter when preparing infusion solution)	7 (days) x 0.09 mg/kg	Bacteriostatic 0.9% Sodium Chloride Injection (0.9% benzyl alcohol)	q.s. to 100 mL

Since limited compatibility data are available, adherence to the recommended diluents and infusion systems is advised. Solutions containing cladribine should not be mixed with other intravenous drugs or additives or infused simultaneously via a common intravenous line, since compatibility testing has not been performed. Preparations containing benzyl alcohol should not be used in neonates. See **WARNINGS**.

Care must be taken to assure the stability of prepared solutions. Once diluted, solutions of cladribine should be administered promptly or stored in the refrigerator (2° to 8° C) for no more than 8 hours prior to start of administration. Vials of cladribine are for single-use only. Any unused portion should be discarded in an appropriate manner. See **Handling and Disposal**.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. A precipitate may occur during the exposure of cladribine to low temperatures; it may be resolubilized by allowing the solution to warm naturally to room temperature and by shaking vigorously. **DO NOT HEAT OR MICROWAVE.**

Chemical Stability of Vials:

When stored in refrigerated conditions between 2° to 8° C (36° to 46° F) protected from light, unopened vials of cladribine are stable until the expiration date indicated on the package. Freezing does not adversely affect the solution. If freezing occurs, thaw naturally to room temperature. **DO NOT** heat or microwave. Once thawed, the vial of cladribine is stable until expiry if refrigerated. **DO NOT** refreeze. Once diluted, solutions containing cladribine should be administered promptly or stored in the refrigerator (2° to 8° C) for no more than 8 hours prior to administration.

Handling and Disposal:

The potential hazards associated with cytotoxic agents are well established and proper precautions should be taken when handling, preparing, and administering cladribine. The use of disposable gloves and protective garments is recommended. If cladribine contacts the skin or mucous membranes, wash the involved surface immediately with copious amounts of water. Several guidelines on this subject have been published.[1-8] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate. Refer to your institution's guidelines and all applicable state/local regulations for disposal of cytotoxic waste.

HOW SUPPLIED

Cladribine Injection is supplied as a sterile, preservative-free, isotonic solution containing 10 mg (1 mg/mL) of cladribine at 10 mL filled into a single-use clear flint glass 20 mL vial, individually boxed. **NDC 55390-124-01**

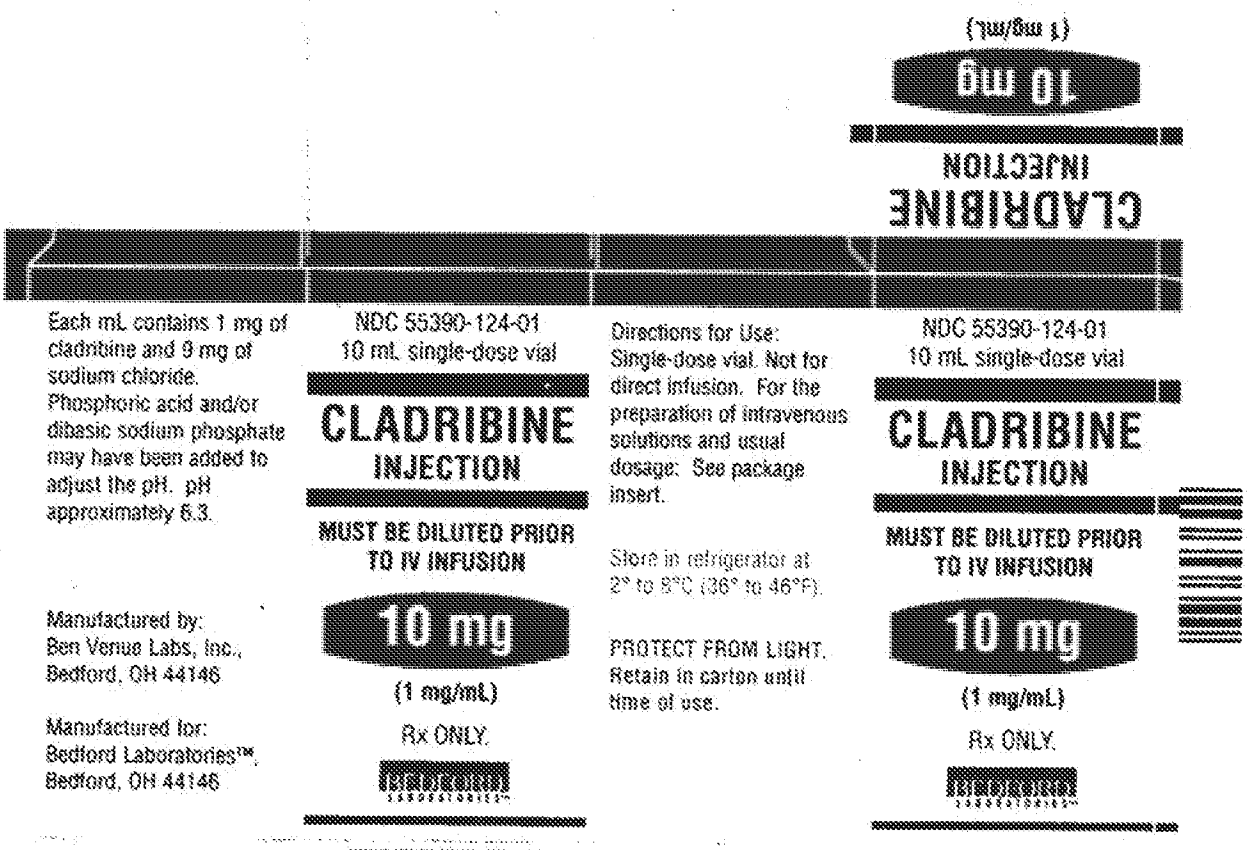
Store refrigerated 2° to 8° C (36° to 46° F). Protect from light during storage.

REFERENCES

1. Santana VM, Mirro J, Harwood FC, *et al.* A Phase I Clinical Trial of 2-Chloro-deoxyadenosine in Pediatric Patients with Acute Leukemia. *J. Clin. Oncol.* 1991; 9: 416.
2. Recommendations for the Safe Handling of Parenteral Antineoplastic Drugs. NIH Publication No. 83-2621. For sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402.
3. AMA Council Report. Guidelines for Handling Parenteral Antineoplastics. *JAMA* 1985; 253 (11): 1590-1592.
4. National Study Commission on Cytotoxic Exposure—Recommendations for Handling Cytotoxic Agents. Available from Louis P. Jeffrey, Chairman, National Study Commission on Cytotoxic Exposure, Massachusetts College of Pharmacy and Allied Health Sciences, 179 Longwood Avenue, Boston, Massachusetts, 02115.
5. Clinical Oncological Society of Australia. Guidelines and Recommendations for Safe Handling of Antineoplastic Agents. *Med J Australia* 1983; 1:426-428.
6. Jones RB, *et al.* Safe Handling of Chemotherapeutic Agents: A Report from the Mount Sinai Medical Center. *CA-A Cancer Journal for Clinicians* 1983; Sept/Oct: 258-263.
7. American Society of Hospital Pharmacists. Technical Assistance Bulletin on Handling Cytotoxic and Hazardous Drugs. *Am J Hosp Pharm* 1990; 47:1033-1049.
8. OSHA Work-Practice Guidelines for Personnel Dealing with Cytotoxic (Antineoplastic) Drugs. *Am J Hosp Pharm* 1986; 43:1193-1204.
9. Viaflex® containers, manufactured by Baxter Healthcare Corporation - Code No. 2B8013 (tested in 1991)
10. MEDICATION CASSETTE™ Reservoir, manufactured by SIMS Deltec, Inc. - Reorder No. 6021/60A (tested in 1991)

Manufactured by:
Ben Venue Laboratories, Inc.
Bedford, OH 44146
January 2000

Manufactured for:
Bedford Laboratories™
Bedford, OH 44146
CLD-P09



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Mark Zarnstorff
Checked by

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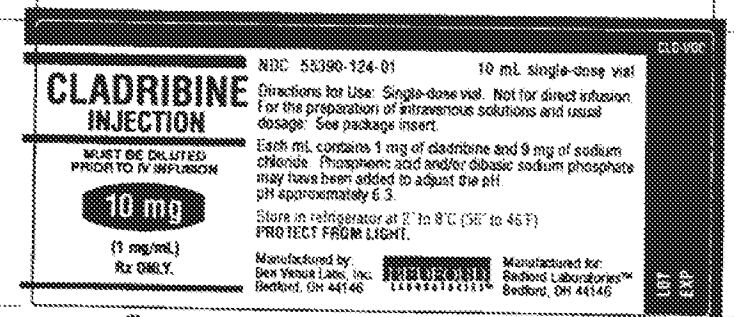

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EXHIBIT U

Drugs@FDA: FDA-Approved Drugs

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Abbreviated New Drug Application (ANDA): 076571
Company: FRESENIUS KABI USA

Products on ANDA 076571

CSV | Excel | Print

Drug Name	Active Ingredients	Strength	Dosage Form/Route	Marketing Status	TE Code	RLD	RS
CLADRIBINE	CLADRIBINE	1MG/ML	INJECTABLE;INJECTION	Prescription	AP	No	Yes

Showing 1 to 1 of 1 entries

Approval Date(s) and History, Letters, Labels, Reviews for ANDA 076571

Therapeutic Equivalents for ANDA 076571

CLADRIBINE

INJECTABLE;INJECTION; 1MG/ML
TE Code = AP

CSV | Excel | Print

Drug Name	Active Ingredients	Strength	Dosage Form/Route	Marketing Status	RLD	TE Code	Application No.
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EXHIBIT V

Drugs@FDA: FDA-Approved Drugs

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PRINT

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Abbreviated New Drug Application (ANDA): 200510
Company: MYLAN LABS LTD

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Products on ANDA 200510

CSV Excel Print

Drug Name	Active Ingredients	Strength	Dosage Form/Route	Marketing Status	TE Code	RLD	RS
CLADRIBINE	CLADRIBINE	1MG/ML	INJECTABLE;INJECTION	Prescription	AP	No	No

Showing 1 to 1 of 1 entries

Approval Date(s) and History, Letters, Labels, Reviews for ANDA 200510

Therapeutic Equivalents for ANDA 200510

CLADRIBINE

INJECTABLE;INJECTION; 1MG/ML
TE Code = AP

CSV Excel Print

Drug Name	Active Ingredients	Strength	Dosage Form/Route	Marketing Status	RLD	TE Code	Application No.
CLADRIBINE	CLADRIBINE	1MG/ML	INJECTABLE;INJECTION	Prescription	No	AP	076571 (/scripts/cder/daf/index.cfm?event=BasicSearch.process&ApplNo=
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CLADRIBINE	CLADRIBINE	1MG/ML	INJECTABLE;INJECTION	Prescription	No	AP	075405 (/scripts/cder/daf/index.cfm?event=BasicSearch.process&ApplNo=

Showing 1 to 4 of 4 entries

Electronic Patent Application Fee Transmittal

Application Number:	11722018
Filing Date:	18-Jun-2007
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Filer:	Eric J.I. Myers/Malika Ash Shakur
Attorney Docket Number:	000758US

Filed as Large Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 5 months with $0 paid	1255	1	3160	3160
Miscellaneous:				
		Total in USD ($)		**3160**

Electronic Acknowledgement Receipt

EFS ID:	44539118
Application Number:	11722018
International Application Number:	
Confirmation Number:	5532
Title of Invention:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Giampiero De Luca
Customer Number:	151167
Filer:	Eric J.I. Myers/Malika Ash Shakur
Filer Authorized By:	Eric J.I. Myers
Attorney Docket Number:	000758US
Receipt Date:	17-DEC-2021
Filing Date:	18-JUN-2007
Time Stamp:	08:12:29
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$3160
RAM confirmation Number	E2021BG912401820
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		2021-12-17-Request-for-Reconsideration-as-filed.pdf	2821751 9450149c82e6be8b2858cd531a0e5cae7236d726	yes	78

Multipart Description/PDF files in .zip description

Document Description	Start	End
Transmittal Letter	1	1
Extension of Time	2	2
37 CFR 1.750 Request for Reconsideration	3	7
Transmittal Letter	8	8
Affidavit-not covered under specific rule	9	40
Affidavit-not covered under specific rule	41	62
Affidavit-not covered under specific rule	63	70
Affidavit-not covered under specific rule	71	74
Affidavit-not covered under specific rule	75	78

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	38411 015422b04f8be3fd65af39fce2289a86d5bad91b	no	2

Warnings:

Information:

Total Files Size (in bytes):	2860162

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

FEE TRANSMITTAL		
	Application Number	11/722,018
	Filing Date	June 18, 2007
	First Named Inventor	Giampiero De Luca
☐No fee required	Art Unit	1649
	Examiner Name	BALLARD, KIMBERLY
☒Total payment $ 3160	Attorney Docket No.	000758US
	Title	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

☐Applicant asserts small entity status, 37 CFR 1.27

☐Applicant asserts micro entity status, 37 CFR 1.29 *(Form PTO/SB/15 or equivalent enclosed or already submitted)*

☐Track 1 Prioritized Examination

Claims Fees:

Total:	(- 20) ×	$100	$
Independent:	(- 3) ×	$480	$
☐Multiple dependency		$860	$
☐Late filing declaration		$160	$
☐Non-electronic filing fee		$400	$
☐Non-English translation		$140	$

Extension Fees under 37 CFR 1.136(a) and 1.17(a), see petition filed herewith, if applicable:

☐Within first month	$ 220	$
☐Within second month	$ 640	$
☐Within third month	$1480	$
☐Within fourth month	$2320	$
☒Within fifth month	$3160	$3160

☐Terminal Disclaimer	$ 170	$
☐RCE – 1st Request	$1360	$
☐RCE – 2nd or Subseq.	$2000	$
☐Notice of Appeal	$ 840	$
☐Appl'n Size (pp.-100)/50 ×	$ 420	$

Other:

☐	$
☐	$
☐	$
☐	$

Payment in the amount of $ 3160 paid by:

☒Credit Card (online if electronically filed, or attached if paper filed)

☐Deposit Account No. **601920**.

☒ Please charge additional fee(s) or underpayment of fee(s) to Deposit Account No. **601920** under 37 CFR 1.16 and 1.17, and please credit any overpayment of fee(s) to Deposit Account No. **601920**.

☒ If these papers are not considered timely, then Applicants hereby petition under 37 CFR 1.136 for any necessary extension of time, further authorizing any necessary extension of time fees to be charged to Deposit Account No. **601920**.

Respectfully Submitted,
GRÜNEBERG AND MYERS PLLC

/Kirsten Grueneberg/

Dr. Kirsten Grueneberg
Registration No. 47,297

Eric Myers
Registration No. 68,546

Customer Number
151167
Phone: (571) 458-7790
Fax: (571) 458-7789

PETITION FOR EXTENSION OF TIME Under 37 CFR 1.136(a)		
	Application Number	11/722,018
	Filing Date	June 18, 2007
	First Named Inventor	Giampiero De Luca
	Art Unit	1649
	Examiner Name	BALLARD, KIMBERLY
	Attorney Docket No.	000758US
	Title	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

Applicants respectfully request that the Office grant an extension of time under 37 CFR 1.136(a) in the above-identified application, for

☐ one month ☐ four months
☐ two months ☒ five months
☐ three months

to December 20, 2021, for filing:

☒ a response to the Office Action (NOTICE OF DETERMINATION OF INELIGIBILITY), mailed May 20, 2021 .
☐ a response to the Notice of Allowability, mailed _____.
☐ a response to the Notice of File Missing Parts, mailed _____.
☐ a Notice of Appeal.
☐ an Appeal Brief, following the Notice of Appeal filed on _____.

Applicants accompany this petition with a Fee Transmittal form to pay the required extension of time fees in accordance with 37 CFR 1.17(a) by credit card (online if electronically filed, or attached if paper filed) or from Deposit Account No. **601920**.

Please charge additional fee(s) or underpayment of fee(s) to Deposit Account No. **601920** under 37 CFR 1.16 and 1.17, and please credit any overpayment of fee(s) to Deposit Account No. **601920**.

Respectfully Submitted,
GRÜNEBERG AND MYERS PLLC

/Kirsten Grueneberg/

Dr. Kirsten Grueneberg
Registration No. 47,297

Eric Myers
Registration No. 68,546

Customer Number
151167
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Docket No. 000758US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT:	U.S. PAT. NO. 7,713,947
ISSUED:	MAY 11, 2010
APPLICATION:	11/722,018
FILED:	JUNE 18, 2007
INVENTORS:	DE LUCA ET AL.
EXPIRATION:	OCTOBER 16, 2026
TITLE:	CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

REQUEST FOR RECONSIDERATION
OF FINAL DETERMINATION OF INELIGIBILITY
ON APPLICATION FOR EXTENSION OF TERM UNDER 35 USC §156

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Commissioner:

Merck Serono SA Switzerland ("Applicant") files the present Request for Reconsideration of the Notice of Determination of Ineligibility ("Notice") dated May 20, 2021 and regarding U.S. Patent No. 7,713,947 ("the '947 patent"). The Notice was in response to an application for Patent Term Extension ("PTE"), which Applicant initially filed for the '947 patent on May 24, 2019.

The Notice provides a two-month period for filing a request for reconsideration, extendable pursuant to 37 C.F.R. 1.136. Applicant hereby petitions for a three-month extension of time under 37 C.F.R. 1.136 and authorizes the United States Patent and Trademark Office ("USPTO") to charge the applicable extension fee, and any additional required fees, to Deposit Account No. 601920.

Applicant respectfully requests reconsideration from the Food and Drug Administration ("FDA") and the USPTO as to whether approval of New Drug Application (NDA) number 22561 qualifies as the first permitted marketing or use of the Approved Product, Mavenclad. Mavenclad contains, among other components, cladribine and hydroxypropyl betadex. (Exhibit H, submitted with the original application for Patent Term Extension for the'947 patent and resubmitted herewith, at pages 19-20). Hydroxypropyl betadex may form complexes. (*Id.* at 23).

As the USPTO has noted, the term of a patent which claims a product shall be extended if, *inter alia*, the permission for the commercial marketing or use of the product is the first permitted commercial marketing or use of the product under the provision of law under which such regulatory review period occurred. (35 U.S.C. §156(a)(5)(A)). The term "product" in the present context extends to "any salt or ester" thereof. (35 U.S.C. §156(f)(1)-(2)). However, the Federal Circuit has found that this broadened definition of the term "product" is limited, and does not extend, for example, to metabolites or de-esterified forms. (*Biogen International GmbH v. Banner Life Sciences*, 956 F. 3d 1351, 1357 (Fed. Cir. 2020)). In addition, for purposes of Patent Term Extension, the relevant product is that which is present in the drug at the time of administration. (*PhotoCure Asa v. Kappos*, 603 F. 3d 1372, 1376 (Fed. Cir. 2010)).

The Notice provides a conclusory statement equating Mavenclad with "cladribine alone," citing the October 13, 2020 letter from the FDA to the USPTO ("FDA Letter"). (Notice at pages 1-2). In turn, the FDA Letter also equates Mavenclad with cladribine, but does not provide supporting analysis or citation for this conclusion. (FDA Letter at page 1). Thus, both the FDA and the USPTO appear to have equated cladribine and Mavenclad without analysis or explanation evidencing a full consideration of the content of Mavenclad.

However, as noted above, the relevant product is that which is present in the drug at the time of administration. As also noted above, Mavenclad contains cladribine and hydroxypropyl betadex. Applicant respectfully submits that the FDA and the USPTO do not appear to have considered the full composition of Mavenclad, as it exists at the time of administration, in stating that the relevant product is "cladribine alone." Reconsideration is respectfully requested.

Moreover, both the Notice and in the FDA letter have concluded that Mavenclad does not represent the first permitted commercial marketing or use of the product based, for example, on a list of four approved formulations that include cladribine. (Notice at page 2; FDA letter at page 1) However, these formulations do not further include hydroxypropyl betadex. First, the Notice and the FDA letter cite the approval of NDA 20229 for Leustatin (cladribine), but the components of this formulation are cladribine, sodium chloride, and optionally phosphoric acid and/or dibasic sodium phosphate. (Exhibit L, submitted with the original application for Patent Term Extension for the'947 patent and resubmitted herewith, at page 1 of the label). Second, the Notice and the FDA letter cite the approval of ANDA 75405, but the components of this formulation are also cladribine, sodium chloride, and optionally phosphoric acid and/or dibasic sodium phosphate. (Exhibit T, "CLADRIBINE INJECTION" (ANDA 75405) label, newly submitted herewith, at page 1). Third and fourth, the Notice and the FDA letter cite the approval of ANDA 76571 and ANDA 200510, but these are both equivalents of ANDA 75405. (Exhibit U, FDA website for ANDA 76571, newly submitted herewith, at page 2; Exhibit V, FDA website for ANDA 200510, newly submitted herewith, at page 2). The FDA and the USPTO further do not appear to have considered whether these products or any other approved products are the same as, or constitute any salt or ester of, the relevant product of Mavenclad, or whether instead the relationship is not one of identity, salt, or ester, but of another type, analogous to metabolites

or de-esterified forms, which would not have constituted the same product for purposes of PTE eligibility.

Thus, the FDA and the USPTO have not provided evidence of any earlier approval of a product containing cladribine with hydroxypropyl betadex.

The question regarding Mavenclad as a first permitted marketing or use under 35 U.S.C. §156(a)(5)(A) appears to have been the sole basis for the dismissal of the PTE application for the'947 patent. (Notice at pages 1-3). Applicant maintains that this and all other requirements under 35 U.S.C. §156 have been satisfied for the grant of PTE as set forth in Applicant's initial PTE application filed May 24, 2019.

Applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

Correspondence relating to the application for patent term extension should be addressed to:

Dr. Kirsten Grüneberg
Grüneberg and Myers, PLLC
1775 Tysons Blvd
5th Floor
Tysons, VA 22102

Telephone: 571-458-7783
Email: patent@gandmpatent.com
Fax: 571-458-7789

* * *

In accordance with the above statements and the exhibits provided herewith, Applicant respectfully requests reconsideration of the Notice and extension of the term of the '947 patent under 35 U.S.C. §156 due to regulatory delay for a period of **1826 days**, as set forth in the original PTE application.

Respectfully Submitted,
GRÜNEBERG AND MYERS PLLC

/Kirsten Grueneberg/

Customer Number
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Fax: (571) 458-7789

Dr. Kirsten Grueneberg
Attorney of Record
Registration No. 47,297

Eric Myers
Registration No. 68,546

LIST OF EXHIBITS

Exhibit	Contents
H	Mavenclad Label (submitted previously and resubmitted herewith)
L	Leustatin (cladribine) NDA 020229: listing and label (submitted previously and resubmitted herewith)
T	"CLADRIBINE INJECTION" (ANDA 75405) label
U	FDA website for ANDA 76571
V	FDA website for ANDA 200510

AO 120 (Rev. 08/10)

TO:	Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☑ Patents (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	7/25/2022	for the District of Delaware

PLAINTIFF	DEFENDANT
MERCK KGaA and MERCK SERONO SA	ACCORD HEALTHCARE, INC. and INTAS PHARMACEUTICALS LTD.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	7,713,947 B2	5/10/2010	Merck Serono SA
2	8,377,903 B2	2/19/2013	Merck Serono SA
3			
4			
5			

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1		
2		
3		
4		
5		

In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE

Copy 1—Upon initiation of action, mail this copy to Director **Copy 3—Upon termination of action, mail this copy to Director**
Copy 2—Upon filing document adding patent(s), mail this copy to Director **Copy 4—Case file copy**