United States Patent And Trademark Office
Patent Trial and Appeal Board

Sandoz Inc.,
Apotex Inc., Apotex Corp., Emcure Pharmaceuticals Ltd., Heritage Pharma Labs Inc., Heritage Pharmaceuticals Inc., Glenmark Pharmaceuticals, Inc., USA, Glenmark Holdings SA, Glenmark Pharmaceuticals, Ltd., Mylan Laboratories Limited, Teva Pharmaceuticals USA, Inc., Fresenius KABI USA, LLC, and Wockhardt Bio AG

Petitioners,

v.

Eli Lilly and Company

Patent Owner

IPR2016-00318[1]
U.S. Patent No. 7,772,209

Petitioner Sandoz Inc.'s Oral Argument Demonstratives

March 16, 2017

[1] Cases IPR2016-01429, IPR2016-01393, and IPR2016-01340 have been joined with the instant proceeding.

Background

OVERVIEW OF GROUNDS

U.S. Patent No. 7,772,209: Institution Grounds

Trials@uspto.gov
571.272.7822

Paper No. 14
Entered: June 16, 2016

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SANDOZ INC.,
Petitioner,

v.

ELI LILLY & COMPANY
Patent Owner.

Case IPR2016-00318
Patent 7,772,209 B2

Before MICHAEL P. TIERNEY, JACQUELINE WRIGHT BONILLA, TINA E. HULSE, *Administrative Patent Judges*.

TIERNEY, *Administrative Patent Judge*.

DECISION
Institution of *Inter Partes* Review
37 C.F.R. § 42.108

Accordingly, it is

ORDERED that pursuant to 35 U.S.C. § 314, an *inter partes* review is hereby instituted as to claims 1–22 of the '209 patent on the following grounds:

References	Basis	Claims challenged
Calvert in view of Niyikiza I, Worzalla, EP 005 and the '974 Patent	§ 103	1–22
Calvert in view of Niyikiza I, Hammond I, EP 005 and the '974 Patent	§ 103	1–22

Paper No. 14, Institution, p.21

U.S. Patent No. 7,772,209: Institution Grounds

Accordingly, it is

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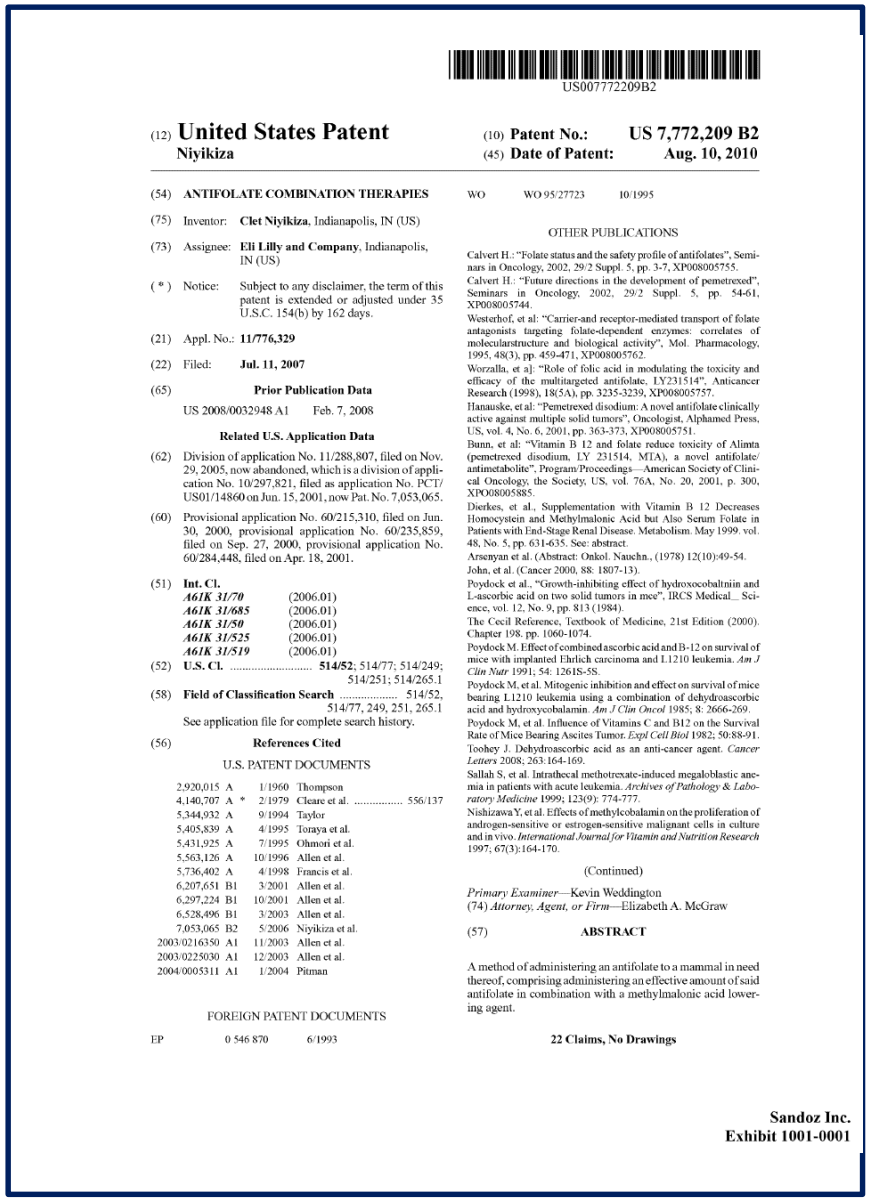
Calvert in view of Niyikiza I, Worzalla, EP 005 and the '974 Patent	§ 103	1–22
Calvert in view of Niyikiza I, Hammond I, EP 005 and the '974 Patent	§ 103	1–22

Paper No. 14, Institution, p.21

- EP005 not admitted at trial or cited by the district court in *Lilly v. Teva et al.* (S.D. Ind.), *aff'd* (Fed. Cir. 2017) (Ex. 1003).

U.S. Patent No. 7,772,209: Overview



US007772209B2

(12) **United States Patent**
Niyikiza

(10) **Patent No.: US 7,772,209 B2**
(45) **Date of Patent: Aug. 10, 2010**

(54) **ANTIFOLATE COMBINATION THERAPIES**

(75) Inventor: **Clet Niyikiza**, Indianapolis, IN (US)

(73) Assignee: **Eli Lilly and Company**, Indianapolis, IN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 162 days.

(21) Appl. No.: **11/776,329**

(22) Filed: **Jul. 11, 2007**

(65) **Prior Publication Data**

US 2008/0032948 A1 Feb. 7, 2008

Related U.S. Application Data

(62) Division of application No. 11/288,807, filed on Nov. 29, 2005, now abandoned, which is a division of application No. 10/297,821, filed as application No. PCT/US01/14860 on Jun. 15, 2001, now Pat. No. 7,053,065.

(60) Provisional application No. 60/215,310, filed on Jun. 30, 2000, provisional application No. 60/235,859, filed on Sep. 27, 2000, provisional application No. 60/284,448, filed on Apr. 18, 2001.

(51) **Int. Cl.**

A61K 31/70	(2006.01)
A61K 31/685	(2006.01)
A61K 31/50	(2006.01)
A61K 31/525	(2006.01)
A61K 31/519	(2006.01)

(52) **U.S. Cl.** **514/52**; 514/77; 514/249; 514/251; 514/265.1

(58) **Field of Classification Search** 514/52, 514/77, 249, 251, 265.1

See application file for complete search history.

(56) **References Cited**

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2,920,015	A		1/1960	Thompson
4,140,707	A	*	2/1979	Cleare et al. 556/137
5,344,932	A		9/1994	Taylor
5,405,839	A		4/1995	Toraya et al.
5,431,925	A		7/1995	Ohmori et al.
5,563,126	A		10/1996	Allen et al.
5,736,402	A		4/1998	Francis et al.
6,207,651	B1		3/2001	Allen et al.
6,297,224	B1		10/2001	Allen et al.
6,528,496	B1		3/2003	Allen et al.
7,053,065	B2		5/2006	Niyikiza et al.
2003/0216350	A1		11/2003	Allen et al.
2003/0225030	A1		12/2003	Allen et al.
2004/0005311	A1		1/2004	Pitman

FOREIGN PATENT DOCUMENTS

EP	0 546 870	6/1993
WO	WO 95/27723	10/1995

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Calvert H.: "Folate status and the safety profile of antifolates", Seminars in Oncology, 2002, 29/2 Suppl. 5, pp. 3-7, XP008005755.

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Bunn, et al: "Vitamin B 12 and folate reduce toxicity of Alimta (pemetrexed disodium, LY 231514, MTA), a novel antifolate/antimetabolite", Program/Proceedings—American Society of Clinical Oncology, the Society, US, vol. 76A, No. 20, 2001, p. 300, XPO08005885.

Dierkes, et al., Supplementation with Vitamin B 12 Decreases Homocystein and Methylmalonic Acid but Also Serum Folate in Patients with End-Stage Renal Disease. Metabolism. May 1999. vol. 48, No. 5, pp. 631-635. See: abstract.

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Poydock M, et al. Influence of Vitamins C and B12 on the Survival Rate of Mice Bearing Ascites Tumor. *Expl Cell Biol* 1982; 50:88-91.

Toohey J. Dehydroascorbic acid as an anti-cancer agent. *Cancer Letters* 2008; 263:164-169.

Sallah S, et al. Intrathecal methotrexate-induced megaloblastic anemia in patients with acute leukemia. *Archives of Pathology & Laboratory Medicine* 1999; 123(9): 774-777.

Nishizawa Y, et al. Effects of methylcobalamin on the proliferation of androgen-sensitive or estrogen-sensitive malignant cells in culture and in vivo. *International Journal for Vitamin and Nutrition Research* 1997; 67(3):164-170.

(Continued)

Primary Examiner—Kevin Weddington
(74) *Attorney, Agent, or Firm*—Elizabeth A. McGraw

(57) **ABSTRACT**

A method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1001-0001

- 22 claims
- 2 independent claims: 1 and 12

Background

U.S. PATENT NO. 7,772,209

U.S. Patent No. 7,772,209: Claims 1 and 12

US007772209B2

(12) **United States Patent**
Niyikiza

(10) **Patent No.: US 7,772,209 B2**
(45) **Date of Patent: Aug. 10, 2010**

(54) **ANTIFOLATE COMBINATION THERAPIES**

(75) Inventor: **Clet Niyikiza**, Indianapolis, IN (US)

(73) Assignee: **Eli Lilly and Company**, Indianapolis, IN (US)

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(51) **Int. Cl.**

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(58) **Field of Classification Search** 514/52, 514/77, 249, 251, 265.1

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(Continued)

Primary Examiner—Kevin Weddington
(74) *Attorney, Agent, or Firm*—Elizabeth A. McGraw

(57) **ABSTRACT**

A method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1001-0001

1. A method for administering pemetrexed disodium to a patient in need thereof comprising administering an effective amount of folic acid and an effective amount of a methylmalonic acid lowering agent followed by administering an effective amount of pemetrexed disodium, wherein

the methylmalonic acid lowering agent is selected from the group consisting of vitamin B12, hydroxycobalamin, cyano-10-chlorocobalamin, aquocobalamin perchlorate, aquo-10-cobalamin perchlorate, azidocobalamin, cobalamin, cyanocobalamin, or chlorocobalamin.

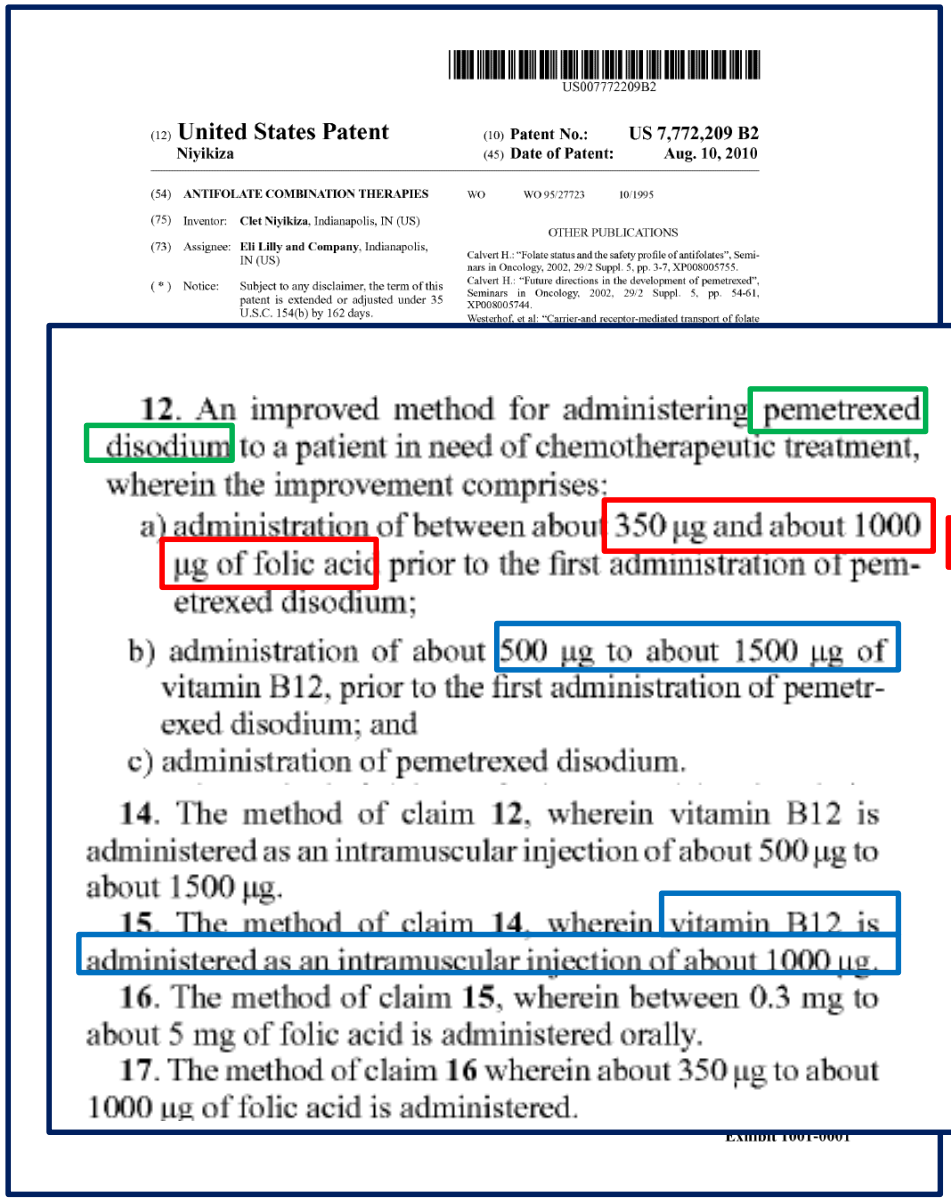
12. An improved method for administering pemetrexed disodium to a patient in need of chemotherapeutic treatment, wherein the improvement comprises:

a) administration of between about 350 μg and about 1000 μg of folic acid prior to the first administration of pemetrexed disodium;

b) administration of about 500 μg to about 1500 μg of vitamin B12, prior to the first administration of pemetrexed disodium; and

c) administration of pemetrexed disodium.

U.S. Patent No. 7,772,209: Claim 12 and its dependents



US007772209B2

(12) **United States Patent**
Niyikiza

(10) **Patent No.: US 7,772,209 B2**
(45) **Date of Patent: Aug. 10, 2010**

(54) **ANTIFOLATE COMBINATION THERAPIES**

(75) Inventor: **Clet Niyikiza**, Indianapolis, IN (US)

(73) Assignee: **Eli Lilly and Company**, Indianapolis, IN (US)

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WO WO 95/27723 10/1995

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Westerhof, et al: "Carrier-and receptor-mediated transport of folate

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b) administration of about 500 μg to about 1500 μg of vitamin B12, prior to the first administration of pemetrexed disodium; and

c) administration of pemetrexed disodium.

14. The method of claim **12**, wherein vitamin B12 is administered as an intramuscular injection of about 500 μg to about 1500 μg.

15. The method of claim **14**, wherein vitamin B12 is administered as an intramuscular injection of about 1000 μg.

16. The method of claim **15**, wherein between 0.3 mg to about 5 mg of folic acid is administered orally.

17. The method of claim **16** wherein about 350 μg to about 1000 μg of folic acid is administered.

18. The method of claim **17** wherein 350 μg to 600 μg of folic acid is administered.

19. The method of claim **18** wherein folic acid is administered 1 to 3 weeks prior to the first administration of the pemetrexed disodium.

20. The method of claim **18** wherein the folic acid is administered from about 1 to about 24 hours prior to administration of the pemetrexed disodium.

21. The method of claim **12**, **18**, or **19**, wherein the vitamin B12 administration is repeated about every 6 to about every 12 weeks following the administration of vitamin B12 until administration of pemetrexed disodium is discontinued.

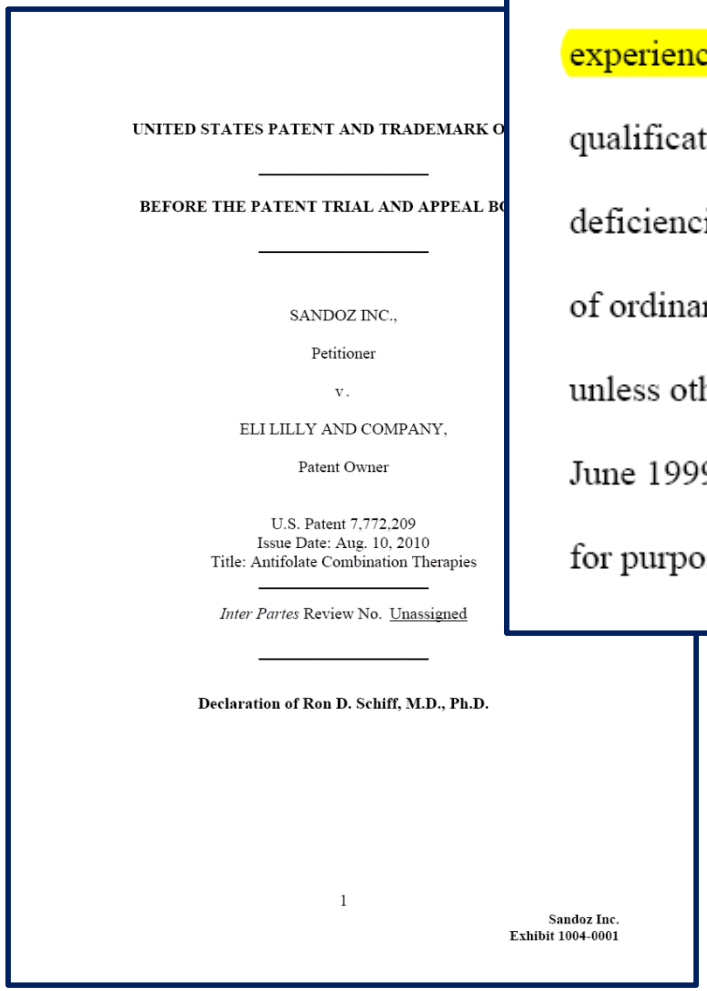
22. The method of claim **21** further comprising the administration of cisplatin to the patient.

Exhibit 1001-0001

Legal Framework

PERSON OF ORDINARY SKILL IN THE ART

Person of Ordinary Skill in the Art



UNITED STATES PATENT AND TRADEMARK O

BEFORE THE PATENT TRIAL AND APPEAL BO

SANDOZ INC.,

Petitioner

v.

ELI LILLY AND COMPANY,

Patent Owner

U.S. Patent 7,772,209
Issue Date: Aug. 10, 2010
Title: Antifolate Combination Therapies

Inter Partes Review No. Unassigned

Declaration of Ron D. Schiff, M.D., Ph.D.

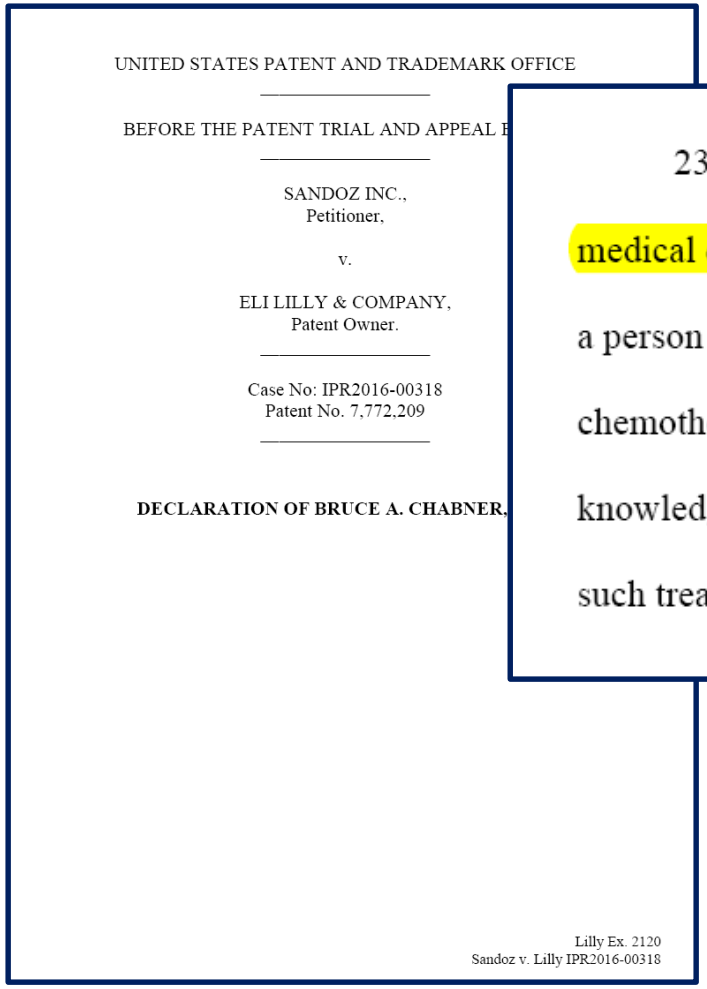
1

Sandoz Inc.
Exhibit 1004-0001

13. The person of ordinary skill in the art ("POSA") would have been a medical doctor experienced in oncology with knowledge of and/or several years of experience regarding the use of antifolates in the treatment of cancer and additional qualifications or experience in the field of nutritional sciences involving vitamin deficiencies. Although my experience and expertise may exceed that of a person of ordinary skill, all opinions and statements made for purposes of this Declaration, unless otherwise noted, reflect the knowledge of the person of ordinary skill as of June 1999, which counsel for Sandoz has informed me is the relevant time period for purposes of my analysis.

Ex. 1004, Schiff Decl. ¶13;
Ex. 1075, Schiff Reply ¶¶7-9

Person of Ordinary Skill in the Art: Lilly Definition



UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL B

SANDOZ INC.,
Petitioner,

v.

ELI LILLY & COMPANY,
Patent Owner.

Case No: IPR2016-00318
Patent No. 7,772,209

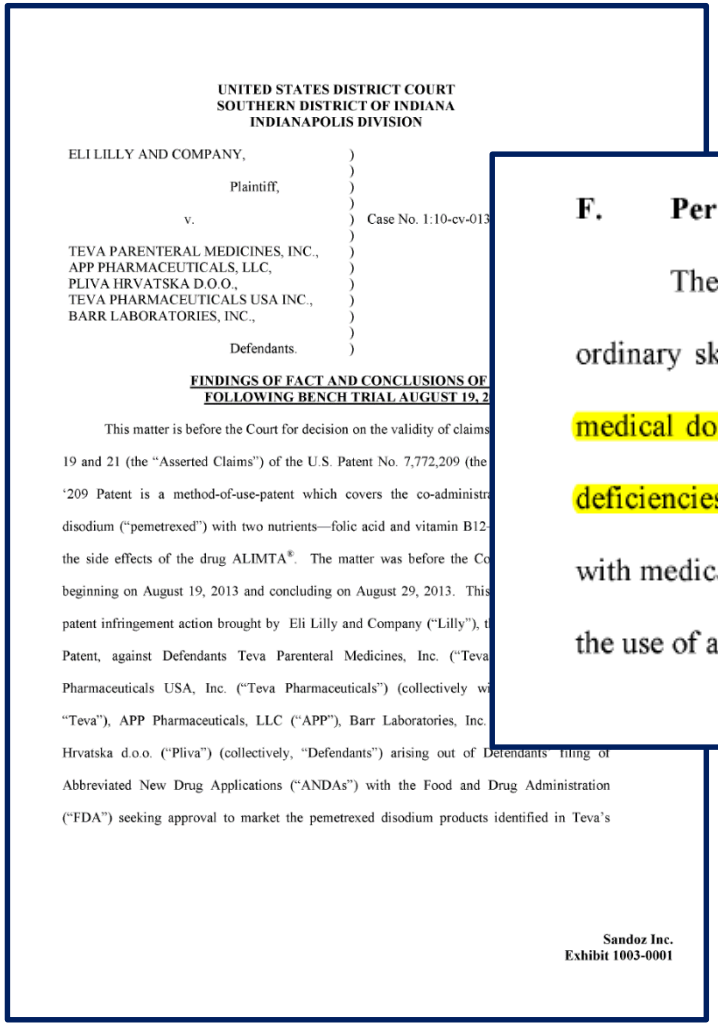
DECLARATION OF BRUCE A. CHABNER,

Lilly Ex. 2120
Sandoz v. Lilly IPR2016-00318

23. In my opinion, the POSA to whom the '209 patent is addressed is a medical doctor who specializes in oncology, specifically medical oncology. Such a person would have knowledge and experience concerning the use of chemotherapy agents, including antifolates, in the treatment of cancer, as well as knowledge and experience regarding the management of toxicities associated with such treatment.

Ex. 2120, Chabner Decl. ¶ 23.

Person of Ordinary Skill in the Art: Lilly v. Teva Definition



UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF INDIANA
INDIANAPOLIS DIVISION

ELI LILLY AND COMPANY,)
)
Plaintiff,)
)
v.) Case No. 1:10-cv-013
)
TEVA PARENTERAL MEDICINES, INC.,)
APP PHARMACEUTICALS, LLC,)
PLIVA HRVATSKA D.O.O.,)
TEVA PHARMACEUTICALS USA INC.,)
BARR LABORATORIES, INC.,)
)
Defendants.)

FINDINGS OF FACT AND CONCLUSIONS OF
FOLLOWING BENCH TRIAL AUGUST 19, 2

This matter is before the Court for decision on the validity of claims 19 and 21 (the "Asserted Claims") of the U.S. Patent No. 7,772,209 (the '209 Patent is a method-of-use-patent which covers the co-administra disodium ("pemetrexed") with two nutrients—folic acid and vitamin B12 the side effects of the drug ALIMTA®. The matter was before the Co beginning on August 19, 2013 and concluding on August 29, 2013. This patent infringement action brought by Eli Lilly and Company ("Lilly"), t Patent, against Defendants Teva Parenteral Medicines, Inc. ("Teva Pharmaceuticals USA, Inc. ("Teva Pharmaceuticals") (collectively wi "Teva"), APP Pharmaceuticals, LLC ("APP"), Barr Laboratories, Inc. Hrvatska d.o.o. ("Pliva") (collectively, "Defendants") arising out of Defendants filing of Abbreviated New Drug Applications ("ANDAs") with the Food and Drug Administration ("FDA") seeking approval to market the pemetrexed disodium products identified in Teva's

Sandoz Inc.
Exhibit 1003-0001

F. Person of Ordinary Skill in the Arts

The Court previously determined, and the parties no longer dispute, that a person of ordinary skill in the art ("POSA") can be a medical doctor who specializes in oncology or a medical doctor with extensive experience in the areas of nutritional sciences involving vitamin deficiencies. However, as to the latter person, this individual would need to have collaborated with medical oncologists who have knowledge and experience in the treatment of cancer through the use of antifolates. *See* Dkt. 115 at 8.

Ex.. 1003, Lilly v. Teva, No. 1:10-cv-1376 (S.D. Ind.), p. 9.

Lilly v. Teva: Southern District of Indiana & Dr. Zeisel

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF INDIANA
INDIANAPOLIS DIVISION

ELI LILLY AND COMPANY,)
)
Plaintiff,)
)
v.) Case No. 1:10-cv-01376-TWP-DKL
)
TEVA PARENTERAL MEDICINES, INC.,)
APP PHARMACEUTICALS, LLC,)
PLIVA HRVATSKA D.O.O.,)
TEVA PHARMACEUTICALS USA INC.,)
BARR LABORATORIES, INC.,)
)
Defendants.)

FINDINGS OF FACT AND CONCLUSIONS OF LAW FOLLOWING BENCH TRIAL AUGUST 19, 2013

This matter is before the Court for decision on the validity of claims 9, 10, 12, 14, 15, 18, 19 and 21 (the "Asserted Claims") of the U.S. Patent No. 7,772,209 (the "'209 Patent"). The '209 Patent is a method-of-use-patent which covers the co-administration of pemetrexed disodium ("pemetrexed") with two nutrients—folic acid and vitamin B12—that protect against the side effects of the drug ALIMTA®. The matter was before the Court for a bench trial beginning on August 19, 2013 and concluding on August 29, 2013. This is a Hatch-Waxman patent infringement action brought by Eli Lilly and Company ("Lilly"), the owner of the '209 Patent, against Defendants Teva Parenteral Medicines, Inc. ("Teva Parenteral"), Teva Pharmaceuticals USA, Inc. ("Teva Pharmaceuticals") (collectively with Teva Parenteral, "Teva"), APP Pharmaceuticals, LLC ("APP"), Barr Laboratories, Inc. ("Barr"), and Pliva Hrvatska d.o.o. ("Pliva") (collectively, "Defendants") arising out of Defendants' filing of Abbreviated New Drug Applications ("ANDAs") with the Food and Drug Administration ("FDA") seeking approval to market the pemetrexed disodium products identified in Teva's

Sandoz Inc.
Exhibit 1003-0001

In addition, neurological damage would have been less of a concern to the POSA treating cancer patients than it would be in treating RA or cardiovascular patients. First, neurotoxicity from B_{12} deficiency, while a potentially serious condition, is very rare, and arguably not a clinical concern for even RA patients. Zeisel Tr. 1387:6-18. Second, neurotoxicity caused by B_{12} deficiency is chronic and progresses slowly over a period of years, while cancer progresses much faster and is lethal if untreated, or if it is not treated effectively. Zeisel Tr. 1577:8-19.

compared to the duration of anti-cancer therapy. Third, cancer patients see their oncologists at very frequent intervals and are monitored for neuropathy among many other potential toxicities, thus there is a very low likelihood that neuropathy would go unnoticed or untreated. Zeisel Tr. 1572:23-1573:20. Finally, it is important to note that the level of "elevation" of homocysteine

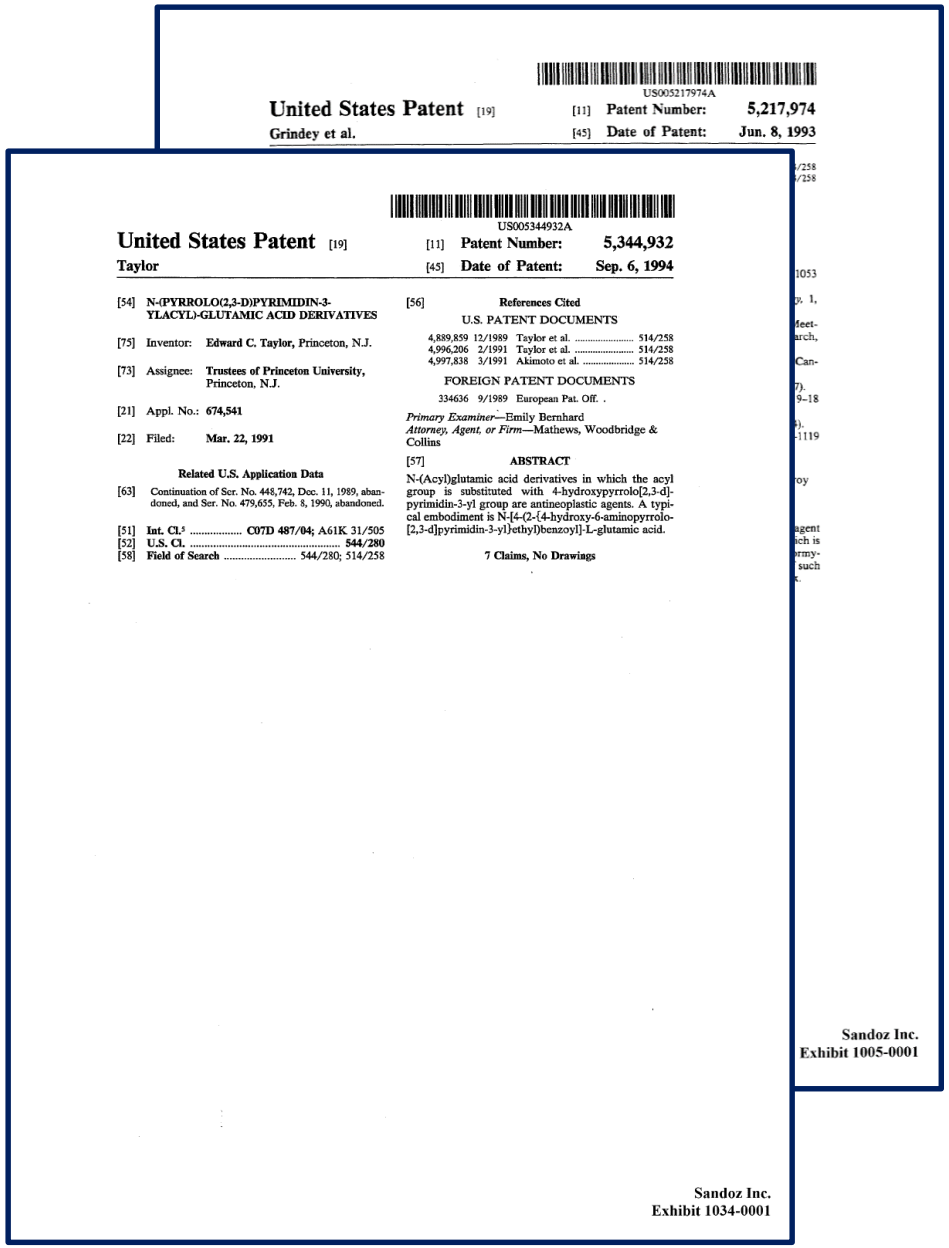
cells. Green Tr. 454:9-17. As of June 1999, the POSA would have expected that vitamin B_{12} would counteract the efficacy of antifolates by increasing the production of a critical folate enzyme, methionine synthase, making more folate available to the cell. TX 2093; Green Tr. 454:5- 455:13; Zeisel Tr. 1617:3-13. Even if a POSA were to conclude from the prior art that

Ex. 1003, Lilly v. Teva (S.D. Ind.), pp. 18-19; *see also id.* at 11-12.

Prior Art Disclosures

PEMETREXED

Prior Art: Pemetrexed



US005217974A

United States Patent [19]
Grindey et al.

[11] Patent Number: 5,217,974
[45] Date of Patent: Jun. 8, 1993

Sandoz Inc.
Exhibit 1005-0001

US005344932A

United States Patent [19]
Taylor

[11] Patent Number: 5,344,932
[45] Date of Patent: Sep. 6, 1994

[54] N-(PYRROLO(2,3-D)PYRIMIDIN-3-YLACYL)-GLUTAMIC ACID DERIVATIVES

[75] Inventor: Edward C. Taylor, Princeton, N.J.

[73] Assignee: Trustees of Princeton University, Princeton, N.J.

[21] Appl. No.: 674,541

[22] Filed: Mar. 22, 1991

Related U.S. Application Data

[63] Continuation of Ser. No. 448,742, Dec. 11, 1989, abandoned, and Ser. No. 479,655, Feb. 8, 1990, abandoned.

[51] Int. Cl.[5] C07D 487/04; A61K 31/505
[52] U.S. Cl. 544/280
[58] Field of Search 544/280; 514/258

[56] References Cited

U.S. PATENT DOCUMENTS

4,889,859 12/1989 Taylor et al. 514/258
4,996,206 2/1991 Taylor et al. 514/258
4,997,838 3/1991 Akimoto et al. 514/258

FOREIGN PATENT DOCUMENTS

334636 9/1989 European Pat. Off. .

Primary Examiner—Emily Bernhard
Attorney, Agent, or Firm—Mathews, Woodbridge & Collins

[57] ABSTRACT

N-(Acyl)glutamic acid derivatives in which the acyl group is substituted with 4-hydroxypyrrolo[2,3-d]-pyrimidin-3-yl group are antineoplastic agents. A typical embodiment is N-[4-(2-{4-hydroxy-6-aminopyrrolo-[2,3-d]pyrimidin-3-yl}ethyl)benzoyl]-L-glutamic acid.

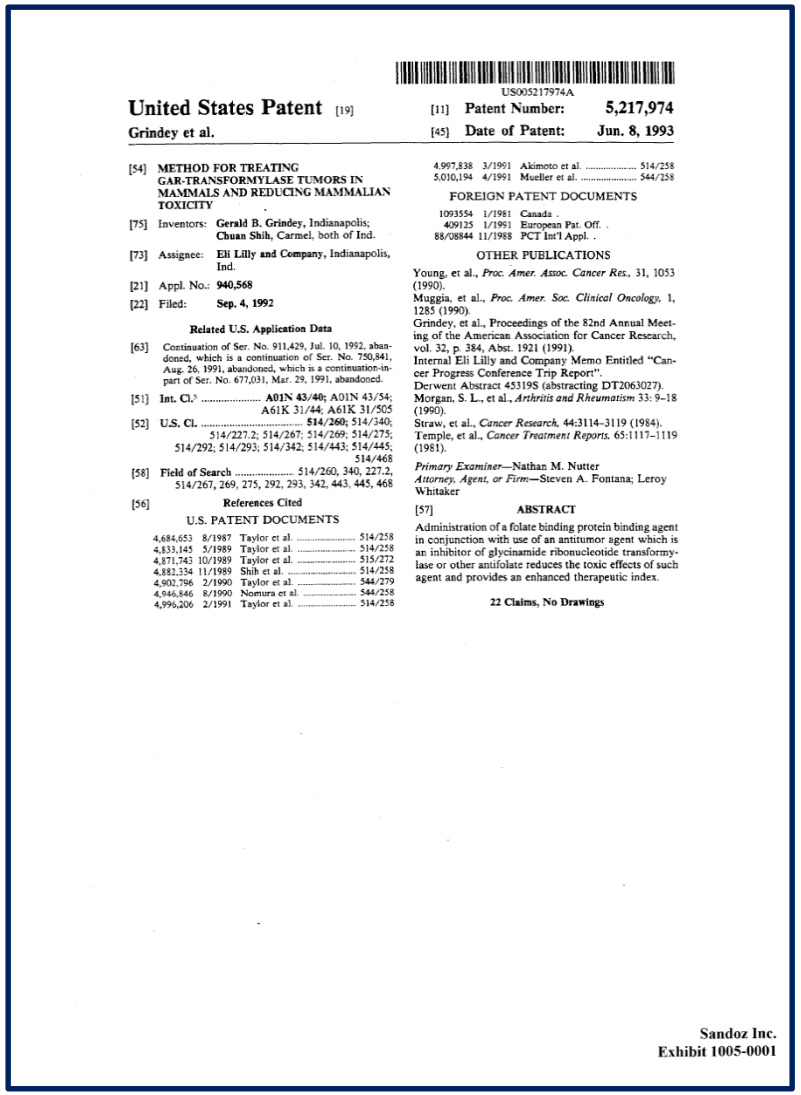
7 Claims, No Drawings

Sandoz Inc.
Exhibit 1034-0001

- Pemetrexed disclosed in U.S. Pat. Nos. 5,217,974 and 5,344,932
- Lilly listed both patents in the Orange Book for pemetrexed/Alimta
- Both the ’932 and ’974 patents are now expired

Exs. 1005, 1025, 1034

Prior Art: ’974 Patent (Ex. 1005) GARFT *or* FBP Binders



US005217974A

United States Patent [19]
Grindey et al.

[11] **Patent Number: 5,217,974**
[45] **Date of Patent: Jun. 8, 1993**

[54] **METHOD FOR TREATING GAR-TRANSFORMYLASE TUMORS IN MAMMALS AND REDUCING MAMMALIAN TOXICITY**

[75] Inventors: **Gerald B. Grindey**, Indianapolis; **Chuan Shih**, Carmel, both of Ind.

[73] Assignee: **Eli Lilly and Company**, Indianapolis, Ind.

[21] Appl. No.: **940,568**

[22] Filed: **Sep. 4, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 911,429, Jul. 10, 1992, abandoned, which is a continuation of Ser. No. 750,841, Aug. 26, 1991, abandoned, which is a continuation-in-part of Ser. No. 677,031, Mar. 29, 1991, abandoned.

[51] **Int. Cl.**[5] **A01N 43/40**; A01N 43/54; A61K 31/44; A61K 31/505

[52] **U.S. Cl.** **514/260**; 514/340; 514/227.2; 514/267; 514/269; 514/275; 514/292; 514/293; 514/342; 514/443; 514/445; 514/468

[58] **Field of Search** 514/260, 340, 227.2, 514/267, 269, 275, 292, 293, 342, 443, 445, 468

[56] **References Cited**

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4,833,145	5/1989	Taylor et al.	514/258
4,871,743	10/1989	Taylor et al.	515/272
4,882,334	11/1989	Shih et al.	514/258
4,902,796	2/1990	Taylor et al.	544/279
4,946,846	8/1990	Nomura et al.	544/258
4,996,206	2/1991	Taylor et al.	514/258
4,997,838	3/1991	Akimoto et al.	514/258
5,010,194	4/1991	Mueller et al.	544/258

FOREIGN PATENT DOCUMENTS

1093554	1/1981	Canada .
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88/08844	11/1988	PCT Int'l Appl. .

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Grindey, et al., Proceedings of the 82nd Annual Meeting of the American Association for Cancer Research, vol. 32, p. 384, Abst. 1921 (1991).
Internal Eli Lilly and Company Memo Entitled "Cancer Progress Conference Trip Report".
Derwent Abstract 45319S (abstracting DT2063027).
Morgan, S. L., et al., *Arthritis and Rheumatism* 33: 9–18 (1990).
Straw, et al., *Cancer Research*, 44:3114–3119 (1984).
Temple, et al., *Cancer Treatment Reports*, 65:1117–1119 (1981).

Primary Examiner—Nathan M. Nutter
Attorney, Agent, or Firm—Steven A. Fontana; Leroy Whitaker

[57] **ABSTRACT**

Administration of a folate binding protein binding agent in conjunction with use of an antitumor agent which is an inhibitor of glycinamide ribonucleotide transformylase or other antifolate reduces the toxic effects of such agent and provides an enhanced therapeutic index.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1005-0001

As noted above, the drug products which can be employed in the present invention include other antifolates which are capable of binding to folate binding protein. Folic acid itself has a binding constant (ng/ml) of 1.8, and lometrexol has a binding constant of 9.7 to bovine FBP. Any GAR-transformylase inhibitor or other antifolate that binds at less than about 500 ng/ml can be utilized in the method of this invention. The

- Pemetrexed is both a GARFT inhibitor and FBP binder:

From the group of TS inhibitors, four compounds were identified for which mFBP has a higher affinity than for FA: LY213514, CB3717, IAHQ, and 2-NH_2-ZD1694. Unlike ob-

Q. -- in June of 1999 would understand that pemetrexed binds to GARFT. Correct?

A. Right. It's actually the polyglutamates that bind to GARFT.

Ex. 1005, ‘974 patent, 4:10-17; Ex. 1022, Westerhof, 463; Ex. 1074, Chabner Dep. 282:4-287:4

Prior Art: ’932 Patent

US005344932A

United States Patent [19]
Taylor

[11] **Patent Number: 5,344,932**
[45] **Date of Patent: Sep. 6, 1994**

[54] **N-(PYRROLO(2,3-D)PYRIMIDIN-3-YLACYL)-GLUTAMIC ACID DERIVATIVES**

[75] Inventor: **Edward C. Taylor,** Princeton, N.J.

[73] Assignee: **Trustees of Princeton University,** Princeton, N.J.

[21] Appl. No.: **674,541**

[22] Filed: **Mar. 22, 1991**

Related U.S. Application Data

[63] Continuation of Ser. No. 448,742, Dec. 11, 1989, abandoned, and Ser. No. 479,655, Feb. 8, 1990, abandoned.

[51] **Int. Cl.**[5] **C07D 487/04;** A61K 31/505
[52] **U.S. Cl.** **544/280**
[58] **Field of Search** 544/280; 514/258

[56] **References Cited**

U.S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

334636 9/1989 European Pat. Off. .

Primary Examiner—Emily Bernhard
Attorney, Agent, or Firm—Mathews, Woodbridge & Collins

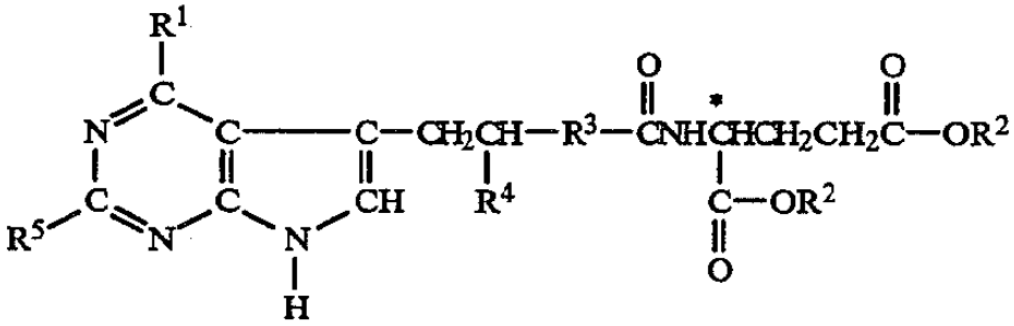
[57] **ABSTRACT**

N-(Acyl)glutamic acid derivatives in which the acyl group is substituted with 4-hydroxypyrrolo[2,3-d]-pyrimidin-3-yl group are antineoplastic agents. A typical embodiment is N-[4-(2-(4-hydroxy-6-aminopyrrolo-[2,3-d]pyrimidin-3-yl}ethyl)benzoyl]-L-glutamic acid.

7 Claims, No Drawings

Sandoz Inc.
Exhibit 1034-0001

1. A compound of the formula:



Pemetrexed:

R^2 is hydrogen or a pharmaceutically acceptable cation:

R^5 is amino; and

2. A compound according to claim 1 wherein R^1 is —OH; R^3 is 1,4-phenylene, and R^4 is hydrogen.

Ex. 1034, claims 1 & 2.

Prior Art: Pemetrexed 2010 Orange Book Listing

APPROVED DRUG PRODUCTS
with
THERAPEUTIC EQUIVALENCE EVALUATIONS

The products in this list have been approved under section 505 of the Federal Food, Drug, and Cosmetic Act. This volume is current through December 31, 2009.

30th EDITION

U.S. DEPARTMENT OF HE
FOOD AND DRU
CENTER FOR DRUG EV
OFFICE OF PHARM
OFFICE OF G

2010

Sandoz Inc.
Exhibit 1025-0001

PRESCRIPTION AND OTC DRUG PRODUCT PATENT AND EXCLUSIVITY LIST

See report footnote for information regarding report content

APPL/PROD NO	PATENT NO	PATENT EXPIRATION DATE	PATENT CODES	PATENT DELIST REQUESTED	EXCLUSIVITY CODE(S)	EXCLUSIVITY EXPIRATION DATE
PEMETREXED DISODIUM - ALIMTA						
N021462 002	5217974	Mar 29, 2011	U-551		I-601	Jul 02, 2012
	5344932	Jul 24, 2016	DS DP		I-571	Sep 26, 2011
					ODE	Feb 04, 2011

Ex. 1025, p. 4

Prior Art: ’974 Patent

US005217974A

United States Patent [19]
Grindey et al.

[11] **Patent Number: 5,217,974**
[45] **Date of Patent: Jun. 8, 1993**

[54] **METHOD FOR TREATING GAR-TRANSFORMYLASE TUMORS IN MAMMALS AND REDUCING MAMMALIAN TOXICITY**

[75] Inventors: **Gerald B. Grindey**, Indianapolis; **Chuan Shih**, Carmel, both of Ind.

[73] Assignee: **Eli Lilly and Company**, Indianapolis, Ind.

[21] Appl. No.: **940,568**

[22] Filed: **Sep. 4, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 911,429, Jul. 10, 1992, abandoned, which is a continuation of Ser. No. 750,841, Aug. 26, 1991, abandoned, which is a continuation-in-part of Ser. No. 677,031, Mar. 29, 1991, abandoned.

[51] **Int. Cl.**[5] **A01N 43/40**; A01N 43/54; A61K 31/44; A61K 31/505

[52] **U.S. Cl.** **514/260**; 514/340; 514/227.2; 514/267; 514/269; 514/275; 514/292; 514/293; 514/342; 514/443; 514/445; 514/468

[58] **Field of Search** 514/260, 340, 227.2, 514/267, 269, 275, 292, 293, 342, 443, 445, 468

[56] **References Cited**

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4,882,334	11/1989	Shih et al.	514/258
4,902,796	2/1990	Taylor et al.	544/279
4,946,846	8/1990	Nomura et al.	544/258
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5,010,194	4/1991	Mueller et al.	544/258

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Internal Eli Lilly and Company Memo Entitled "Cancer Progress Conference Trip Report".
Derwent Abstract 45319S (abstracting DT2063027).
Morgan, S. L., et al., *Arthritis and Rheumatism* 33: 9–18 (1990).
Straw, et al., *Cancer Research*, 44:3114–3119 (1984).
Temple, et al., *Cancer Treatment Reports*, 65:1117–1119 (1981).

Primary Examiner—Nathan M. Nutter
Attorney, Agent, or Firm—Steven A. Fontana; Leroy Whitaker

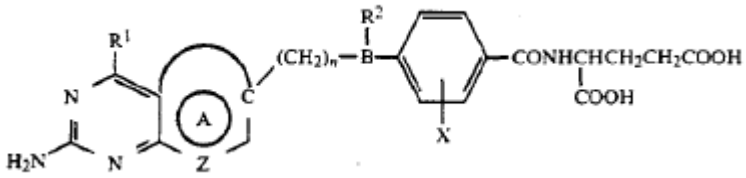
[57] **ABSTRACT**

Administration of a folate binding protein binding agent in conjunction with use of an antitumor agent which is an inhibitor of glycinamide ribonucleotide transformylase or other antifolate reduces the toxic effects of such agent and provides an enhanced therapeutic index.

22 Claims, No Drawings

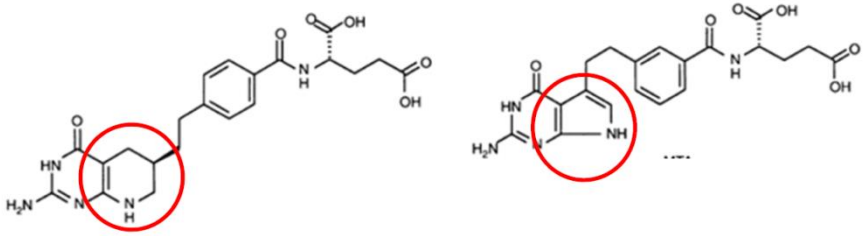
Sandoz Inc.
Exhibit 1005-0001

In a preferred embodiment of the invention, folic acid is administered to a subject subsequently receiving an agent defined by the formula



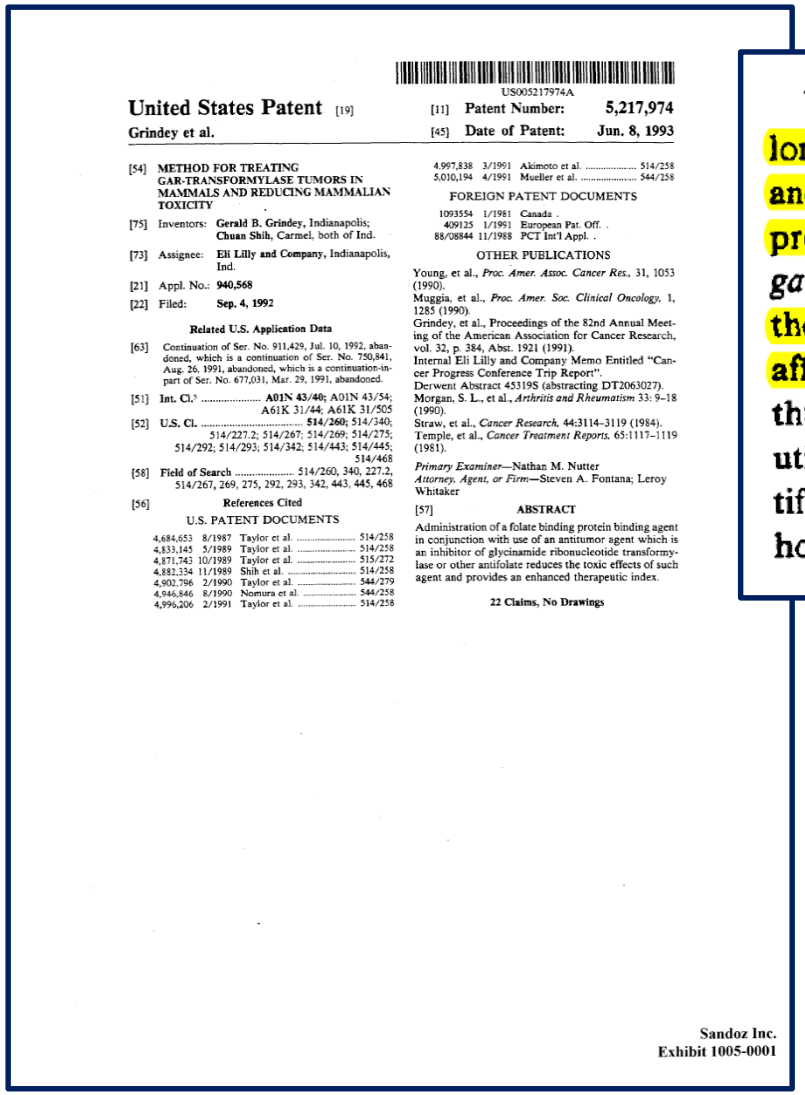
wherein
R^1 is hydroxy or amino;
R^2 is hydrogen, methyl, ethyl, or propynyl;
B is —CH— or —N—;
n is 1, 2 or 3;
Z is nitrogen or carbon;
A is pyrido, tetrahydropyrido, pyrrolo, dihydropyrrolo, cyclopentyl or cyclohexyl;
X is hydrogen or halo; and pharmaceutically acceptable salts thereof.

Lometrexol **Pemetrexed**



Ex. 1005, col. 2, l. 66-col. 3, l. 19; Ex. 1004, Schiff ¶¶ 34, 47; Ex. 1075, Schiff Reply, ¶ 61.

Prior Art: ’974 Patent



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United States Patent [19]
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[54] **METHOD FOR TREATING GAR-TRANSFORMYLASE TUMORS IN MAMMALS AND REDUCING MAMMALIAN TOXICITY**

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[58] **Field of Search** 514/260, 340, 227.2, 514/267, 269, 275, 292, 293, 342, 443, 445, 468

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Administration of a folate binding protein binding agent in conjunction with use of an antitumor agent which is an inhibitor of glycinamide ribonucleotide transformylase or other antifolate reduces the toxic effects of such agent and provides an enhanced therapeutic index.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1005-0001

We have now discovered that the toxic effects of lometrexol and related GAR-transformylase inhibitors and other antifolate agents which bind to folate binding protein (FBP) (see, e.g., Kane, et al., *Laboratory Investigation*, 60, 737 (1989)) can be significantly reduced by the presence of a FBP binding agent, without adversely affecting therapeutic efficacy. The present invention thus provides a method for improving the therapeutic utility of GAR-transformylase inhibitors and other antifolates by co-administering a FBP binding agent to the host under going treatment.

Q. And the patent states that the teachings apply to things that inhibit GARFT or bind folate-binding protein agent. Right?

A. I don't think it says "or." Does it say "or"? Does it say "and"? Oh, it does say "or." So "binds to the folate-binding protein" would be covered.

Ex. 1005, ‘974 patent, 1:47-58; Ex. 1074, Chabner Dep. 282:4-287:4; *See also,* Ex. 1075, Schiff Reply, ¶ 61.

Prior Art: Shih (Ex. 1021)

[CANCER RESEARCH 57, 1116–1123, March 15, 1997]

LY231514, a Pyrrolo[2,3-d]pyrimidine-based Antifolate That Inhibits Multiple Folate-requiring Enzymes

Chuan Shih,[1] Victor J. Chen, Lynn S. Gossett, Susan B. Gates, Warren C. MacKellar, Lillian L. Habeck, Katherine A. Shackelford, Lurane G. Mendelsohn, Daniel J. Soose, Vinod F. Patel, Sherri L. Andis, Jesse R. Bewley, Elizabeth A. Rayl, Barbara A. Moroson, G. Peter Beardsley, William Kohler, Manshan Ratnam, and Richard M. Schultz

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285 [C. S., V. J. C., L. S. G., S. B. G., W. C. M., L. L. H., K. A. S., L. G. M., D. J. S., V. F. P., S. L. A., J. R. B., R. M. S.]; Department of Pediatrics, Yale University, New Haven, Connecticut 06510 [E. A. R., B. A. M., G. P. B.]; and Department of Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, Ohio 43699 [W. K., M. R.]

ABSTRACT

***N*-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-ethyl]-benzoyl]-L-glutamic acid (LY231514) is a novel pyrrolo[2,3-d]pyrimidine-based antifolate currently undergoing extensive Phase II clinical trials. Previous studies have established that LY231514 and its synthetic γ-polyglutamates (glu_3 and glu_5) exert potent inhibition against thymidylate synthase (TS). We now report that LY231514 and its polyglutamates also markedly inhibit other key folate-requiring enzymes, including dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyltransferase (GARFT). For example, the K_i values of the pentaglutamate of LY231514 are 1.3, 7.2, and 65 nM for inhibition against TS, DHFR, and GARFT, respectively. In contrast, although a similar high level of inhibitory potency was observed for the parent monoglutamate against DHFR (7.0 nM), the inhibition constants (K_i) for the parent monoglutamate are significantly weaker for TS (109 nM) and GARFT (9,300 nM). The effects of LY231514 and its polyglutamates on aminoimidazole carboxamide ribonucleotide formyltransferase, 5,10-methylenetetrahydrofolate dehydrogenase, and 10-formyltetrahydrofolate synthetase were also evaluated. The end product reversal studies conducted in human cell lines further support the concept that multiple enzyme-inhibitory mechanisms are involved in cytotoxicity. The reversal pattern of LY231514 suggests that although TS may be a major site of action for LY231514 at concentrations near the IC_{50}, higher concentrations can lead to inhibition of DHFR and/or other enzymes along the purine *de novo* pathway. Studies with mutant cell lines demonstrated that LY231514 requires polyglutamation and transport via the reduced folate carrier for cytotoxic potency. Therefore, our data suggest that LY231514 is a novel classical antifolate, the antitumor activity of which may result from simultaneous and multiple inhibition of several key folate-requiring enzymes via its polyglutamated metabolites.**

INTRODUCTION

Several novel folate-based antimetabolites are currently being actively investigated in clinical trials. These include lometrexol and LY309887,[2] which inhibit GARFT in the purine *de novo* biosynthetic pathway (1–3); edatrexate (4, 5) which acts on DHFR; and ZD1694 (Tomudex; Refs. 6 and 7), AG337 (Thymitaq; Ref. 8), and BW1843U89 (9), which specifically target TS.

LY231514 is a structurally novel antifolate antimetabolite that possesses the unique 6-5-fused pyrrolo[2,3-d]pyrimidine nucleus (10, 11) instead of the more common 6-6-fused pteridine or quinazoline ring structure (Fig. 1). Previous studies have demonstrated that LY231514 is one of the best substrates that is known for the enzyme FPGS (K_m = 1.6 μM and V_{max}/K_m = 621; Ref. 12). It is likely that polyglutamation and the polyglutamated metabolites of LY231514 play profound roles in determining both the selectivity and the antitumor activity of this novel agent (11, 12). Whereas LY231514 only moderately inhibited TS (K_i = 340 nM, recombinant mouse), the pentaglutamate of LY231514 was 100-fold more potent (K_i = 3.4 nM; Ref. 11), making LY231514 one of the most potent folate-based TS inhibitors known today (13).

Preliminary cell culture end product reversal studies in human CCRF-CEM and murine L1210 leukemia cells have demonstrated that thymidine (5 μM) alone was not able to fully reverse the cytotoxic action of LY231514 (11). Both thymidine (5 μM) and hypoxanthine (100 μM) were required to fully protect cells from the growth-inhibitory activity exerted by LY231514. This reversal pattern is significantly different from other TS inhibitors, such as ZD1694 (6) and BW1843U89 (9). Cell culture experiments showed that the antiproliferative activity of LY231514 was completely reversed by the addition of leucovorin (0.05–16 μM) in a competitive manner (11), suggesting that LY231514 competed with natural reduced folate cofactors both at transport and intracellular folate levels and acted as a pure folate antagonist.

Promising antitumor responses have recently been observed in the Phase I trials of LY231514. Moreover, patients who had previously failed to respond to ZD1694 and 5-fluorouracil/leucovorin treatment responded to LY231514 (14). This pattern of clinical response, together with the aforementioned observations of partial protection by thymidine in cell culture, suggest that inhibition of TS by LY231514 may not solely account for the overall antitumor effect of this novel antifolate. LY231514 and its polyglutamates may inhibit other folate-requiring enzymes, such as DHFR, or enzymes along the *de novo* purine biosynthetic pathway. LY231514 may thus act as a multitargeted antifolate, with multiple mechanisms of action affecting the intracellular folate pools and cellular pyrimidine/purine biosynthesis.

We now summarize our findings of LY231514 and its polyglutamates (glu_3 and glu_5) against various folate-requiring enzymes, including human TS, DHFR, AICARFT, 5,10-methylenetetrahydrofolate dehydrogenase, and 10-formyltetrahydrofolate synthetase activities of C1-S and murine GARFT. In addition, we report a detailed comparison of cell culture reversal patterns observed in several human cell lines between compounds LY231514 and ZD1694. Finally, we examine the role of polyglutamation and transport (via the RFC) in the cytotoxicity of LY231514.

MATERIALS AND METHODS

Materials. LY231514 and ZD1694 were prepared according to published methods and procedures (7, 11). The syntheses of the γ-glutamyl derivatives of LY231514 were by the method of Pawelczak *et al.* (15). For *in vitro* studies,

Received 8/21/96; accepted 1/17/97.

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[1] To whom requests for reprints should be addressed, at Cancer Research Division, Lilly Research Laboratories, Drop 0540, Eli Lilly and Company, 307 E. McCarty St., Indianapolis, IN 46285. Phone: (317) 276–3520; Fax: (317) 277–3652.

[2] The abbreviations used are: LY231514, *N*-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]-benzoyl]-L-glutamic acid; *r*, recombinant; h, human; m, murine; TS, thymidylate synthase (EC 2.1.1.45); DHFR, dihydrofolate reductase (EC 1.5.1.3); GARFT, glycinamide ribonucleotide formyltransferase (EC 2.1.2.2); AICA, 5-aminoimidazole-4-carboxamide; AICARFT, aminoimidazole carboxamide ribonucleotide formyltransferase (EC 2.1.2.3); C1-S, C1 tetrahydrofolate synthase; FPGS, folylpolyglutamate synthetase (EC 6.3.2.17); RFC, reduced folate carrier; FBP-α, folate binding protein-α; MTT, 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide; NADPH, β-NADP$^+$, reduced form; 6R-MTHF, 6[R]-5,10-methylene-5,6,7,8-tetrahydrofolate.

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Sandoz Inc.
Exhibit 1021-0001

ABSTRACT

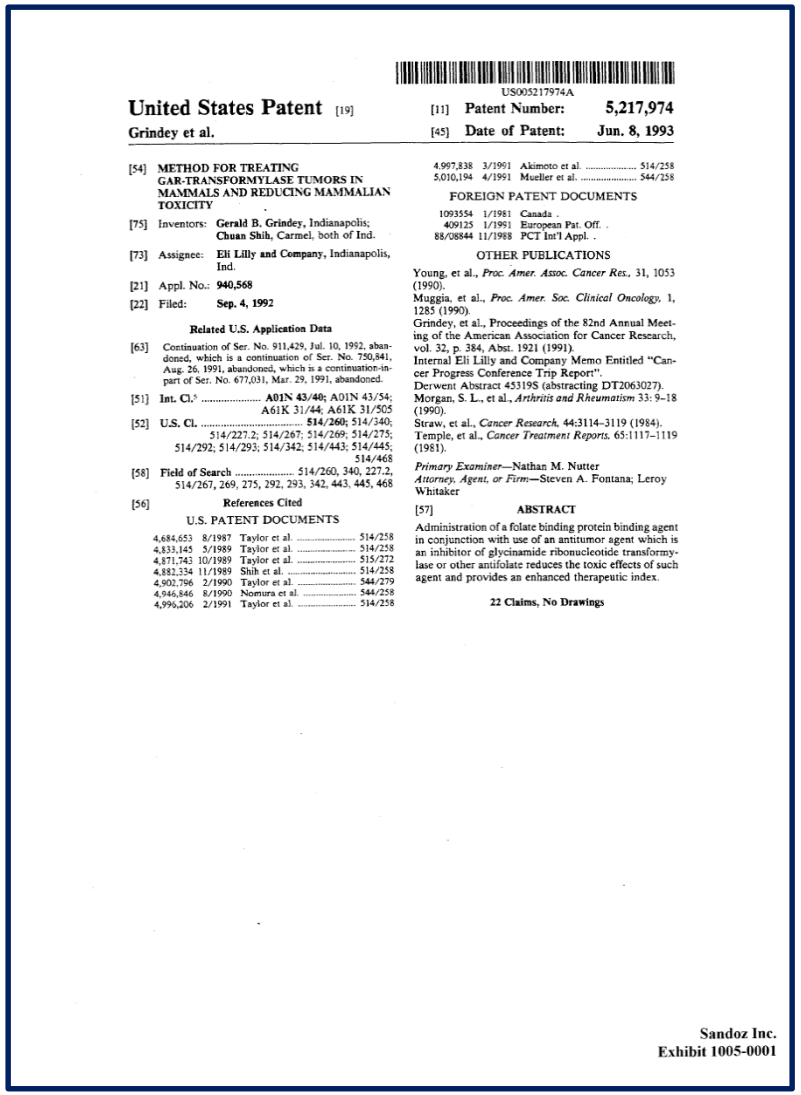
***N*-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-ethyl]-benzoyl]-L-glutamic acid (LY231514) is a novel pyrrolo[2,3-d]pyrimidine-based antifolate currently undergoing extensive Phase II clinical trials. Previous studies have established that LY231514 and its synthetic γ-polyglutamates (glu_3 and glu_5) exert potent inhibition against thymidylate synthase (TS). We now report that LY231514 and its polyglutamates also markedly inhibit other key folate-requiring enzymes, including dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyltransferase (GARFT). For example, the K_i values of the pentaglutamate of LY231514 are 1.3, 7.2, and 65 nM for inhibition against TS, DHFR, and GARFT, respectively. In contrast, although a similar high level of inhibitory potency was observed for the parent monoglutamate against DHFR (7.0 nM), the inhibition constants (K_i) for the parent monoglutamate are significantly weaker for TS (109 nM) and GARFT (9,300 nM). The effects of LY231514 and its polyglutamates on aminoimidazole carboxamide ribonucleotide formyltransferase, 5,10-methylenetetrahydrofolate dehydrogenase, and 10-formyltetrahydrofolate synthetase were also evaluated. The end product reversal studies conducted in human cell lines further support the concept that multiple enzyme-inhibitory mechanisms are involved in cytotoxicity. The reversal pattern of LY231514 suggests that although TS may be a major site of action for LY231514 at concentrations near the IC_{50}, higher concentrations can lead to inhibition of DHFR and/or other enzymes along the purine *de novo* pathway. Studies with mutant cell lines demonstrated that LY231514 requires polyglutamation and transport via the reduced folate carrier for cytotoxic potency. Therefore, our data suggest that LY231514 is a novel classical antifolate, the antitumor activity of which may result from simultaneous and multiple inhibition of several key folate-requiring enzymes via its polyglutamated metabolites.**

Ex. 1021, Shih, p. 1116; Ex. 1004, Schiff Decl. ¶33

Prior Art Disclosures

PEMETREXED + FOLIC ACID PRETREATMENT

Prior Art: ’974 Patent



US005217974A

United States Patent [19]
Grindey et al.

[11] **Patent Number: 5,217,974**
[45] **Date of Patent: Jun. 8, 1993**

[54] **METHOD FOR TREATING GAR-TRANSFORMYLASE TUMORS IN MAMMALS AND REDUCING MAMMALIAN TOXICITY**

[75] Inventors: **Gerald B. Grindey**, Indianapolis; **Chuan Shih**, Carmel, both of Ind.

[73] Assignee: **Eli Lilly and Company**, Indianapolis, Ind.

[21] Appl. No.: **940,568**

[22] Filed: **Sep. 4, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 911,429, Jul. 10, 1992, abandoned, which is a continuation of Ser. No. 750,841, Aug. 26, 1991, abandoned, which is a continuation-in-part of Ser. No. 677,031, Mar. 29, 1991, abandoned.

[51] **Int. Cl.**[5] **A01N 43/40**; A01N 43/54; A61K 31/44; A61K 31/505

[52] **U.S. Cl.** **514/260**; 514/340; 514/227.2; 514/267; 514/269; 514/275; 514/292; 514/293; 514/342; 514/443; 514/445; 514/468

[58] **Field of Search** 514/260, 340, 227.2, 514/267, 269, 275, 292, 293, 342, 443, 445, 468

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Primary Examiner—Nathan M. Nutter
Attorney, Agent, or Firm—Steven A. Fontana; Leroy Whitaker

[57] **ABSTRACT**

Administration of a folate binding protein binding agent in conjunction with use of an antitumor agent which is an inhibitor of glycinamide ribonucleotide transformylase or other antifolate reduces the toxic effects of such agent and provides an enhanced therapeutic index.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1005-0001

antifolate. Although one single dose of the FBP binding agent, preferably an oral administration of folic acid, should be sufficient to load the folate binding protein, multiple dosing of the FBP binding agent can be employed for periods up to weeks before treatment with the active agent to ensure that the folate binding protein is sufficiently bound in order to maximize the benefit derived from such pretreatment.

In the especially preferred embodiment of this invention, about 1 mg to about 5 mg of folic acid is administered orally to a mammal about 1 to about 24 hours prior to the parenteral administration of the amount of lomotrexol which is normally required to attain the desired therapeutic benefit. Although greater or addi-

Ex. 1005, col. 6, ll. 29-43; Ex. 1004, Schiff ¶¶ 112-14; Ex. 1075, Schiff Reply, ¶ 119-21.

Prior Art: Hammond Abstracts (Ex. 1014, 1015)

620P **A phase I and pharmacokinetic (PK) study of the multitargeted antifolate (MTA, LY231514) with folic acid (FA)**

L. Hammond[1], M. Villalona-Calero[1], S.G. Eckhardt[1], L. Siu[1], M. Hidalgo[1], D. Thornton[2], J. Walling[2], S. Baker, C. Coltman[1], D. Von Hoff[1], E. Rowinsky[1]. [1]*Cancer Therapy and Research Center, San Antonio, TX, and* [2]*Eli Lilly, Indianapolis, IN, USA*

Introduction: MTA, a new antifolate that inhibits thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase, demonstrated notable broad antitumour activity when infused 10 min i.v. every 21 days. Myelosuppression precluded dose escalation above 500–600 mg/m^2. As preclinical evaluations indicate that FA supplementation increases the therapeutic index of MTA, this study was initiated to determine if FA supplementation permits significant dose-escalation above the recommended phase II dose of MTA alone. Vitamin metabolites were measured to determine their value as potential prognostic markers with this combination.

Methods: So far, 33 minimally- and heavily-pretreated pts received 90 courses of FA (5 mg/day) for 5 days starting 2 days before MTA at 600, 700, 800 925 mg/m^2. Vitamin metabolites were evaluated during cycles 1 and 2 as potential determinants of principal toxicities and effects.

Results: Principal drug-related toxicities include neutropenia, anaemia and thrombocytopenia, which were more severe in heavily-pretreated pts. Other toxicities (grade (G) 1–2) include rash, somnolence, fatigue, leg oedema, and a decrease in creatinine clearance (CrCl). Severe toxicities in 2 pts, 1 who had taken a non steroidal anti-inflammatory agent and 1 with severe hypoalbuminaemia, resolved after administration of leucovorin and thymidine. Preliminary vitamin metabolites in 26 pts reveal: 2 and 3 of 11 pts with homocysteine ≥ 10 had G4 thrombocytopenia and neutropenia, respectively; 1 and 2 of 15 pts with homocysteine < 10 had G4 thrombocytopenia and neutropenia, respectively; 1 and 2 of 9 pts with elevated cystathionine levels (cystathionine upper limit of normal 342 nM/L) had G2 somnolence and G1–2 fatigue, respectively; 1 and 10 of 16 pts with normal cystathionine levels had G2 somnolence and G1–2 fatigue, respectively; 1 of 4 pts with elevated methylmalonic acid (methylmalonic acid upper limit of normal 271 nM/L) had G2 fatigue while 12 of 22 pts with normal levels had G1–2 fatigue. 7 of 15 pts with elevated homocysteine, cystathionine, or methylmalonic acid levels had a significant decrease in CrCl. Based on information from these 15 pts, addition of FA may reduce the usefulness of vitamin metabolites as predictors of toxicity.

Conclusions: FA supplementation appears to permit MTA dose escalation by ameliorating toxicity. Heavily- and minimally-pretreated pts tolerate MTA at 700 and 925 mg/m^2 and accrual continues at 800 and 925 mg/m^2, respectively.

Ex. 1015, Hammond I (ESMO); Ex. 1004, Schiff ¶¶54, 56; Ex. 1075, Schiff Reply, ¶36.

*866

A PHASE I AND PHARMACOKINETIC (PK) STUDY OF THE MULTITARGETED ANTIFOL (MTA) LY231514 WITH FOLIC ACID. *L. Hammond, M. Villalona-Calero, S.G. Eckhardt, R. Drengler, C. Aylesworth, T. Johnson, M. Hidalgo, G. Rodriguez, S. Diab, P. Monroe, D. Thornton, D. Von Hoff, and E. Rowinsky. Cancer Therapy and Research Center and Brooke Army Med Center, San Antonio, TX, and Eli Lilly Company, Indianapolis, IN.*

MTA (LY 231514) is a new antifol that inhibits multiple folate-dependent enzymes, including thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase. Initial phase I trials demonstrated major antitumor responses when MTA was given as a 10 min i.v. infusion, however, myelosuppression precluded dose escalation above 500–600 mg/m^2. Since preclinical studies indicated that folic acid supplementation increases the therapeutic index of MTA, the feasibility of administering folic acid 5 mg daily for 5 days starting 2 days before MTA in minimally- and heavily-pretreated pts was evaluated to determine if folic acid supplementation ameliorates the toxic effects of MTA, permitting significant dose-escalation above the recommended phase II dose of MTA alone. Thus far, 21 pts with solid cancers have received 55 courses at the following dose levels: 600, 700, and 800 mg/m^2. Drug-related toxicities have included neutropenia, anemia, and thrombocytopenia, which have been more severe in heavily-pretreated pts. Other toxicities (grade 1–2) include rash, somnolence, fatigue, leg edema, and diminished renal function manifested by a decrease in creatinine clearance. One pt taking a non-steroidal anti-inflammatory agent experienced severe toxicities at the 800 mg/m^2 dose, which resolved after administration of leucovorin and thymidine. One partial response in a pt with metastatic colon cancer has been observed. PK and vitamin (folic acid) metabolite profiles were done during cycles 1 and 3 at 600 and 800 mg/m^2. To date, serum folic acid levels do not appear to be related to toxicity, but homocysteine was significantly elevated in the pt with severe toxicities at the 800 mg/m^2 dose. Thus far, heavily- and minimally-pretreated patients have tolerated MTA at 600 and 800 mg/m^2 and accrual continues at 700 and 900 mg/m^2, respectively. These results indicate that folic acid supplementation appears to permit MTA dose escalation.

Ex. 1014, Hammond II (ASCO); Ex. 1004, Schiff ¶¶55-56; Ex. 1075, Schiff Reply, ¶36.

Dr. Chabner's Hammond v. Rinaldi Comparison

Hammond Abstracts, Ex. 1014, 1015

620P A phase I and pharmacokinetic (PK) study of the multitargeted antifolate (MTA, LY231514) with folic acid (FA)

L. Hammond[1], M. Villalona-Calero[1], S.G. Eckhardt[1], L. Siu[1], M. Hidalgo[1], D. Thornto[1]

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Introducti... hydrofolate... demonstra... ery 21 da... mg/m². As... the therap... mentation... ll dose of... value as p... Method... courses of... 800 925 m... potential d... Results... thrombocy... toxicities (... and a dec... who had ... hypoalbum... Preliminar... homocyste... 1 and 2 c... neutropeni... (cystathion... fatigue, re... had G2 sc... methylmal... G2 fatigue... pts with e... had a sign... addition of... of toxicity. Conclu... by amelior... 700 and 92...

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A PHASE I AND PHARMACOKINETIC (PK) STUDY OF THE MULTITARGETED ANTIFOL (MTA) LY231514 WITH FOLIC ACID. *L. Hammond, M. Villalona-Calero, S.G. Eckhardt, R. Drengler, C. Aylesworth, T. Johnson, M. Hidalgo, G. Rodriguez, S. Diab, P. Monroe, D. Thornton, D. Von Hoff, and E. Rowinsky. Cancer Therapy and Research Center and Brooke Army Med Center, San Antonio, TX, and Eli Lilly Company, Indianapolis, IN.*

MTA (LY 231514) is a new antifol that inhibits multiple folate-dependent enzymes, including thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase. Initial phase I trials demonstrated major antitumor responses when MTA was given as a 10 min i.v. infusion, however, myelosuppression precluded dose escalation above 500–600 mg/m². Since preclinical studies indicated that folic acid supplementation increases the therapeutic index of MTA, the feasibility of administering folic acid 5 mg daily for 5 days starting 2 days before MTA in minimally- and heavily-pretreated pts was evaluated to determine if folic acid supplementation ameliorates the toxic effects of MTA, permitting significant dose-escalation above the recommended phase II dose of MTA alone. Thus far, 21 pts with solid cancers have received 55 courses at the following dose levels: 600, 700, and 800 mg/m². Drug-related toxicities have included neutropenia, anemia, and thrombocytopenia, which have been more severe in heavily-pretreated pts. Other toxicities (grade 1–2) include rash, somnolence, fatigue, leg edema, and diminished renal function manifested by a decrease in creatinine clearance. One pt taking a non-steroidal anti-inflammatory agent experienced severe toxicities at the 800 mg/m² dose, which resolved after administration of leucovorin and thymidine. One partial response in a pt with metastatic colon cancer has been observed. PK and vitamin (folic acid) metabolite profiles were done during cycles 1 and 3 at 600 and 800 mg/m². To date, serum folic acid levels do not appear to be related to toxicity, but homocysteine was significantly elevated in the pt with severe toxicities at the 800 mg/m² dose. Thus far, heavily- and minimally-pretreated patients have tolerated MTA at 600 and 800 mg/m² and accrual continues at 700 and 900 mg/m², respectively. These results indicate that folic acid supplementation appears to permit MTA dose escalation.

Rinaldi Abstract, Ex. 2022

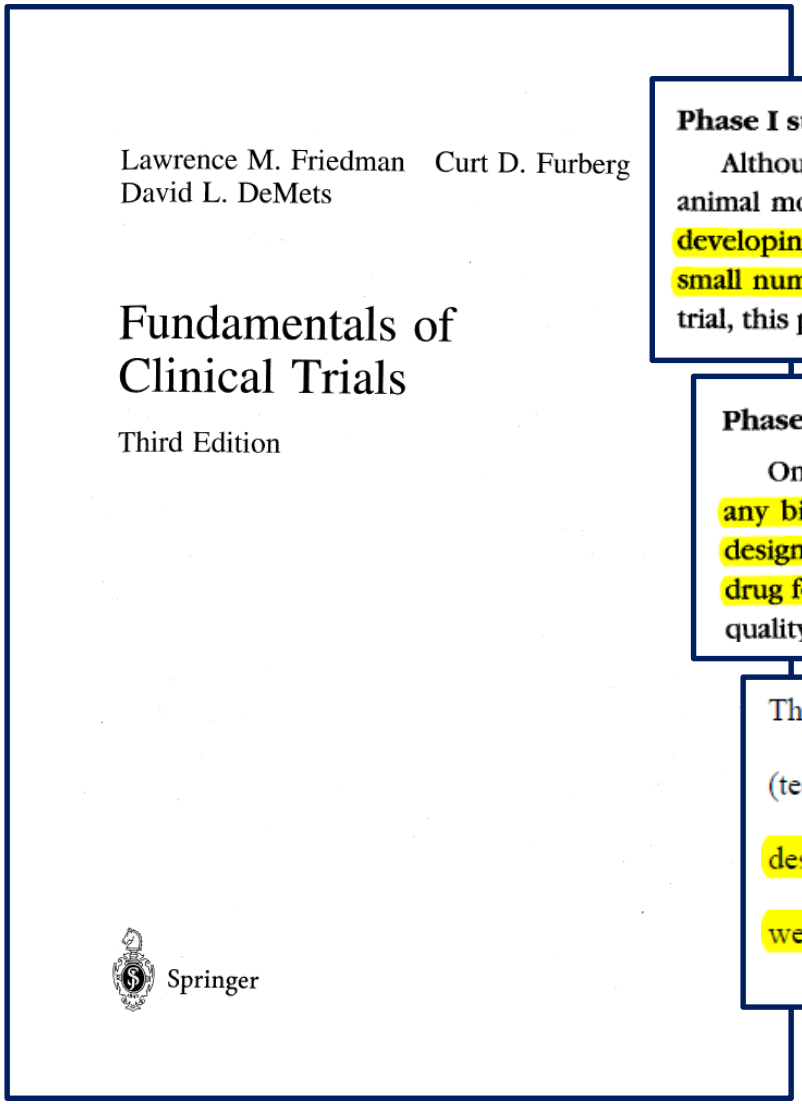
A PHASE I EVALUATION OF LY231514, A NOVEL MULTI-TARGETED ANTIFOLATE, ADMINISTERED EVERY 21 DAYS. DA Rinaldi, HA Burris, FA Dorr, G Rodriguez, SG Eckhardt, SM Fields, JR Woodworth, JG Kuhn, C Langley, G Clark, P Lu, DD Von Hoff. From the Cancer Therapy and Research Center and Brooke Army Medical Center, San Antonio, TX, and Eli Lilly and Company, Indianapolis, IN.

LY231514 (N-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo,3-d] pyrimidin -5-yl) ethyl] benzoyl]-L-glutamic acid disodium salt) is a multi-targeted antifolate compound which inhibits the enzymes thymidylate synthase and dihydrofolate reductase. Escalating doses were administered intravenously every 21 days to patients with advanced, refractory, solid tumors to assess toxicities and determine the maximally-tolerated dose (MTD), pharmacokinetic profile, and potential antitumor activity of the compound. Dose escalation was based on the Modified Continual Reassessment Method, with 1 patient treated at each minimally toxic dose level. A total of 37 patients (27 males, 10 females, median age 59 yo, median PS 90%) were treated with 132 courses at 9 dose levels, ranging from 50 to 700 mg/m². The MTD of LY231514 was 600 mg/m², with reversible neutropenia, thrombocytopenia, and fatigue as the dose-limiting toxicities. Nonhematologic toxicities observed included mild to moderate fatigue, anorexia, nausea, diarrhea, mucositis, rash, and reversible hepatic transaminase elevations. Pharmacokinetic analysis after the first course of treatment at the 600 mg/m² dose level demonstrated a mean harmonic half-life, maximum plasma concentration, clearance, area under the curve (AUC), and apparent volume of distribution at steady-state of 5.07 hours, 142 mcg/ml, 41.7 ml/min/m², 293.3 mcg-hr/ml., and 26.55 L/m² respectively. Seventy-eight percent of the compound was excreted unchanged in the urine. Partial responses were achieved in 2 patients with advanced pancreatic cancer and in 2 patients with advanced colorectal cancer. Minor responses were obtained in 6 patients with advanced colorectal cancer. LY231514 is a promising agent for the treatment of gastrointestinal malignancies.

1. Cannot quantify efficacy in Phase 1
2. Phase 1 trials lack randomized design or control groups
3. A POSA would not consider Hammond to be a Phase 1B study

Ex. 1075, Schiff Reply, ¶¶37-45.

Flaw #1: Efficacy Cannot be Quantified in Phase I Trials



Phase I studies

Although useful preclinical information may be obtained from in vitro studies or animal models, early data must be obtained in humans. The first step, or phase in developing a drug or a biologic is to understand how well it can be tolerated in a small number of individuals. Although it does not meet our definition of a clinical trial, this phase is commonly called a phase I trial. People who participate in phase I

Phase II studies

Once the MTD is established, the next goal is to evaluate whether the drug has any biologic activity or effect and to estimate the rate of adverse events. If the design of the phase I trial has not been adequate, the investigator may evaluate the drug for activity at too low or high a dose. Thus, the phase II design depends on the quality and adequacy of the phase I study. The results of the phase II trial will, in

The POSA would recognize that the Hammond study was a phase I study (technically a phase IB study). At the relevant date, phase I studies were not designed to demonstrate whether the regimen used was efficacious, that is, they were not statistically powered to give precise information about efficacy.

Ex. 1095, Friedman, 3-4; Ex. 2120, Chabner Decl, ¶ 72;
see also, Ex. 1075, Schiff Reply, ¶¶ 37-45.

Flaw #2: The Hammond and Rinaldi Phase I Trials Have Different Experimental Conditions

Ex. 1015, Hammond I:

Methods: So far, 33 minimally- and heavily-pretreated pts received 90 courses of FA (5 mg/day) for 5 days starting 2 days before MTA at 600, 700, 800 925 mg/m^2. Vitamin metabolites were evaluated during cycles 1 and 2 as potential determinants of principal toxicities and effects.

Ex. 1014, Hammond II:

alone. Thus far, 21 pts with solid cancers have received 55 courses at the following dose levels: 600, 700, and 800 mg/m^2. Drug-related toxicities

Ex. 2022, Rinaldi I:

LY231514 (N-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo,3-d] pyrimidin -5-yl) ethyl] benzoyl]-L-glutamic acid disodium salt) is a multi-targeted antifolate compound which inhibits the enzymes thymidylate synthase and dihydrofolate reductase. Escalating doses were administered intravenously every 21 days to patients with advanced, refractory, solid tumors to assess toxicities and determine the maximally-tolerated dose (MTD), pharmacokinetic profile, and potential antitumor activity of the compound. Dose escalation was based on the Modified Continual Reassessment Method, with 1 patient treated at each minimally toxic dose level. A total of 37 patients (27 males, 10 females, median age 59 yo, median PS 90%) were treated with 132 courses at 9 dose levels, ranging from 50 to 700 mg/m^2. The MTD of LY231514 was 600 mg/m^2, with reversible

Flaw #3: A POSA would not consider Hammond to be a Phase 1B study

620P **A phase I and pharmacokinetic (PK) study of the multitargeted antifolate (MTA, LY231514) with folic acid (FA)**

L. Hammond[1], M. Villalona-Calero[1], S.G. Eckhardt[1], L. Siu[1], M. Hidalgo[1], D. Thornton[2], J. Walling[2], S. Baker, C. Coltman[1], D. Von Hoff[1], E. Rowinsky[1]. [1]*Cancer Therapy and Research Center, San Antonio, TX, and* [2]*Eli Lilly, Indianapolis, IN, USA*

2562 **General Poster Session (Board #C4), Sun, 8:00 AM - 12:00 PM**

The complete phase Ib clinical trial: A method to accelerate new agent development. *D. D. Von Hoff, J. A. Nieves, L. K. Vocila, S. D. Weitman, E. Cvitkovic; Translational Genomics Research Institute (TGen), Phoenix, AZ; Translational Oncology Team, Houston, TX; Institute for Drug Development, San Antonio, TX; AAI Oncology, Le Kremlin, France*

Background: The usual clinical development plan for a new agent (NA) includes a phase I monotherapy trial. However, because many new agents are eventually developed as part of a combination, additional phase I trials assessing the new agent in combination with a standard agent are usually performed (usually as individual phase Ib studies). Our hypothesis was that within a single protocol, several combination phase I trials could be conducted simultaneously. **Meth**ing the new agent is synergistic w agent, an antimetabolite, an a EGFR): patients with advanced deemed most likely to be of help-with a choice of a) anthracycline + NA; b) tubulin interactive + NA; c) antimetabolite + NA; d) angiogenesis inhibitor + NA; e) antibody to EGFR + NA. The standard agent is started at full dose with 3 patients placed at 1/3 full dose of NA, 3 patients at 2/3 dose of NA, and 3–6 patients at full dose of the NA in the combination. **Results:** Our experience with the complete phase Ib trial has found several advantages over conducting separate phase I trials. The advantages include: (a) a very rapid follow-up on preclinical data in one study; (b) a saving of time and expense in the start up; (c) accrual is rapid because many patients in a practice are likely to be eligible (e.g. the standard agent is the standard of care); (d) patients are often less pretreated; (e) the trial generates information for more informed selection of follow-up randomized phase II or III trials. **Conclusions:** Utilizing a Complete phase Ib trial design is feasible. Our initial experience has suggested that this approach is safe and highly efficient with several potential advantages over multiple sequential combination phase Ib studies.

Ex. 1098, Von Hoff, *see also,* Ex. 1075, Schiff Reply, ¶ 44.

Flaw #3: A POSA would not consider Hammond to be a Phase 1B study

Lilly Clinical Trial Website 2001:

Phase I A Phase I clinical trial is the first step in testing a new investigational medication in humans. Phase I studies are mainly concerned with how the drug is absorbed and broken down by the body. These studies help determine the best way to give a drug to a patient (for example by mouth, or by injection), and what side effects may be likely. Except for drugs used to treat cancer, Phase I clinical trials are usually conducted in healthy individuals and are not intended to treat disease or illness. Because cancer can be such a life-threatening condition, Phase I trials with anti-cancer drugs are usually carried out in patients who already have the disease.

Phase II Phase II clinical trials may involve up to several hundred volunteers who have the disease state or condition to be treated. These trials may last one to two years as physicians and researchers begin to learn more about the safety of the new drug treatment and how well it treats the disease or condition. Several different doses of the drug may be looked at to see which dose has the desired effects. Patients are watched for side effects and for any improvement in their illness, symptoms, or both.

Lilly Clinical Trial Website 2006:

- **Phase 1** : A Phase 1 clinical trial is the first step in testing a new investigational medication (or new use of a marketed drug) in humans. Phase 1 studies are mainly concerned with evaluating a drug's safety profile, including the safe dosage range. The studies also determine how the drug is absorbed and broken down by the body, what is the best way to give the drug to a patient (for example by mouth, or by injection), what side effects may be likely, and how the drug is absorbed, distributed, metabolized, and excreted as well as its duration of action. Except for drugs used to treat cancer, Phase 1 clinical trials are usually conducted in healthy individuals and are not intended to treat disease or illness. Because cancer can be such a life-threatening condition, Phase 1 trials with anti-cancer drugs are usually carried out in patients who already have the disease.
- **Phase 1b** : Phase 1b studies are usually conducted in patients diagnosed with the disease, or condition for which the study drug is intended, who demonstrate some biomarker, surrogate, or possibly clinical outcome that could be considered for "proof of concept." Proof of concept in a Phase 1b study typically confirms the hypothesis that the current prediction of biomarker, or outcome benefit is compatible with the mechanism of action.

Ex. 1082, 11, 15; Ex. 1075, Schiff Reply, ¶ 44.

Dr. Chabner's Alleged Concerns About Kidney Toxicity from Hammond Dose Escalation

and a decrease in creatinine clearance (CrCl)."). A POSA simply would not have been concerned about grade II creatinine clearance, particularly given the high doses of pemetrexed administered in the Hammond dose escalation studies. I note also that Dr. Chabner's claim that a POSA would consider this grade II kidney toxicity a "significant issue" (Ex. 2120, Chabner Decl. ¶ 80) cannot be reconciled with his views that grade III and IV toxicities would not have been viewed as a problem. (*See* Ex. 2120, Chabner Decl. ¶¶ 53-60.)

Ex. 1075, Schiff Reply, ¶51

Prior Art: Laohavinij (Ex. 2031)

Investigational New Drugs **14:** 325–335, 1996.
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A Phase I clinical study of the antipurine antifolate lometrexol (DDATHF) given with oral folic acid

Sudsawat Laohavinij,* Stephen R. Wedge*, Micheal J. Lind, Nigel Bailey, Alison Humphreys, Madeleine Proctor, Fiona Chapman, Dorothy Simmons, Avril Oakley, Lesley Robson, Lyndsey Gumbrell, Gordon A. Taylor, Huw D. Thomas, Alan V. Boddy, David R. Newell and A. Hilary Calvert
Cancer Research Unit, The Medical School, University of Newcastle-upon-Tyne, Framlington Place, Newcastle-upon-Tyne, NE2 4HH, UK

Key words: lometrexol, lometrexol-toxicity, lometrexol-clinical efficacy, lometrexol and folic acid, DDATHF

Summary

Lometrexol is an antifolate which inhibits glycinamide ribonucleotide formyltransferase (GARFT), an enzym essential for *de novo* purine synthesis. Extensive experimental and limited clinical data have shown that lometrexo has activity against tumours which are refractory to other drugs, notably methotrexate. However, the initial clinica development of lometrexol was curtailed because of severe and cumulative antiproliferative toxicities.

Preclinical murine studies demonstrated that the toxicity of lometrexol can be prevented by low dose folic aci administration, i.e. for 7 days prior to and 7 days following a single bolus dose. This observation prompted Phase I clinical study of lometrexol given with folic acid supplementation which has confirmed that the toxicit of lometrexol can be markedly reduced by folic acid supplementation. Thrombocytopenia and mucositis were th major toxicities. There was no clear relationship between clinical toxicity and the extent of plasma folate elevatio

Associated studies demonstrated that lometrexol plasma pharmacokinetics were not altered by folic acid admi istration indicating that supplementation is unlikely to reduce toxicity by enhancing lometrexol plasma clearance

The work described in this report has identified for the first time a clinically acceptable schedule for th administration of a GARFT inhibitor. This information will facilitate the future evaluation of this class of compound in cancer therapy.

Introduction

Lometrexol (5,10-dideaza-5,6,7,8-tetrahydrofolate-(6R)-DDATHF) is a new folate analogue which was synthesized in 1985 by Taylor and colleagues [1]. Unlike methotrexate, lometrexol does not inhibit dihydrofolate reductase, but acts instead against glycinamide ribonucleotide formyltransferase (GARFT), an enzyme essential for *de novo* purine synthesis [2]. Both *in vitro* and *in vivo*, lometrexol has been shown to have antitumor activity against murine and human tumour cells [2–4], and on the basis of its preclinical activit was selected for clinical trial.

In previous Phase I studies of lometrexol when gi en alone, the total dose of lometrexol which cou be safely given was found to be only 10–12 mg/m *per* course [5–7]. In marked contrast, in mice, 60 mg/m^2/week was tolerated in chronic toxicity studie [8]. Furthermore, the onset of profound myelosuppre sion and/or mucositis in most patients 6–8 weeks afte lometrexol administration prevented administration more than two courses of therapy in most studies. Thu it has not been possible to perform Phase II studies evaluate the potential efficacy of lometrexol. Howe er, evidence of antitumor activity was observed in t Phase I clinical studies of lometrexol, in patients wi

* Supported by Eli Lilly and Company, Indianapolis, IN, USA. Financial support was also provided by the North of England Cancer Research Campaign.

Lilly Ex. 2031
Sandoz v. Lilly IPR2016-00318

In summary, the work described in this report has demonstrated that lometrexol toxicity can be modulated by folic acid supplementation in patients. The information obtained from both preclinical murine, and the clinical Phase I study of lometrexol with folate supplementation reported here, indicates that the MTD of lometrexol given with folate supplementation may be higher than the current dose level. The mechanism responsible for the reduction in lometrexol toxicity has not been defined, although associated pharmacokinetic studies suggest that folic acid is not acting by enhancing lometrexol plasma clearance [12]. This work has identified for the first time a safe and acceptable clinical schedule for the administration of a GARFT inhibitor, and the information obtained from this study will facilitate the future development and evaluation of this class of compounds in the treatment of human cancer.

Ex. 2031, Laohavinij at 333-34; Ex. 1075, Schiff Reply ¶¶ 46-50

Prior Art: Laohavinij (Ex. 2031)

Investigational New Drugs **14:** 325–335, 1996.
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A Phase I clinical study of the antipurine antifolate lometrexol (DDATHF) given with oral folic acid

Sudsawat Laohavinij,* Stephen R. Wedge*, Micheal J. Lind, Nigel Bailey, Alison Humphreys, Madeleine Proctor, Fiona Chapman, Dorothy Simmons, Avril Oakley, Lesley Robson, Lyndsey Gumbrell, Gordon A. Taylor, Huw D. Thomas, Alan V. Boddy, David R. Newell and A. Hilary Calvert
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Key words: lometrexol, lometrexol-toxicity, lometrexol-clinical efficacy, lometrexol and folic acid, DDATH

Summary

Lometrexol is an antifolate which inhibits glycinamide ribonucleotide formyltransferase (GARFT), an enzyme essential for *de novo* purine synthesis. Extensive experimental and limited clinical data have shown that lometrexol has activity against tumours which are refractory to other drugs, notably methotrexate. However, the initial clinical development of lometrexol was curtailed because of severe and cumulative antiproliferative toxicities.

Preclinical murine studies demonstrated that the toxicity of lometrexol can be prevented by low dose folic acid administration, i.e. for 7 days prior to and 7 days following a single bolus dose. This observation prompted a Phase I clinical study of lometrexol given with folic acid supplementation which has confirmed that the toxicity of lometrexol can be markedly reduced by folic acid supplementation. Thrombocytopenia and mucositis were the major toxicities. There was no clear relationship between clinical toxicity and the extent of plasma folate elevation.

Associated studies demonstrated that lometrexol plasma pharmacokinetics were not altered by folic acid administration indicating that supplementation is unlikely to reduce toxicity by enhancing lometrexol plasma clearance.

The work described in this report has identified for the first time a clinically acceptable schedule for the administration of a GARFT inhibitor. This information will facilitate the future evaluation of this class of compounds in cancer therapy.

Introduction

Lometrexol (5,10-dideaza-5,6,7,8-tetrahydrofolate-(6R)-DDATHF) is a new folate analogue which was synthesized in 1985 by Taylor and colleagues [1]. Unlike methotrexate, lometrexol does not inhibit dihydrofolate reductase, but acts instead against glycinamide ribonucleotide formyltransferase (GARFT), an enzyme essential for *de novo* purine synthesis [2]. Both *in vitro* and *in vivo*, lometrexol has been shown to have antitumor activity against murine and human tumour cells [2–4], and on the basis of its preclinical activity was selected for clinical trial.

In previous Phase I studies of lometrexol when given alone, the total dose of lometrexol which could be safely given was found to be only 10–12 mg/m^2 *per* course [5–7]. In marked contrast, in mice, 600 mg/m^2/week was tolerated in chronic toxicity studies [8]. Furthermore, the onset of profound myelosuppression and/or mucositis in most patients 6–8 weeks after lometrexol administration prevented administration of more than two courses of therapy in most studies. Thus, it has not been possible to perform Phase II studies to evaluate the potential efficacy of lometrexol. However, evidence of antitumor activity was observed in the Phase I clinical studies of lometrexol, in patients with

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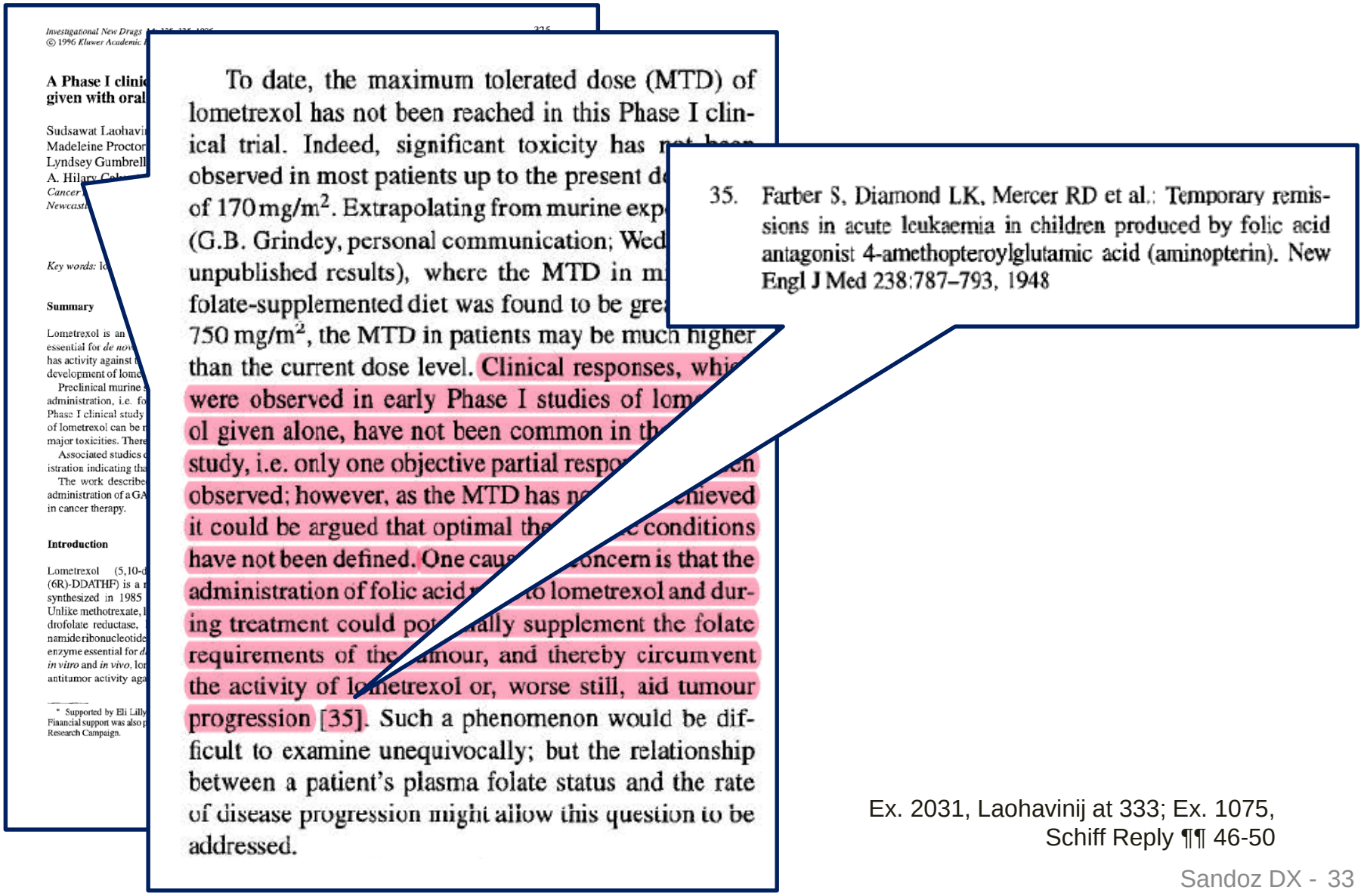
Lilly Ex. 2031
Sandoz v. Lilly IPR2016-00318

To date, the maximum tolerated dose (MTD) of lometrexol has not been reached in this Phase I clinical trial. Indeed, significant toxicity has not been observed in most patients up to the present dose level of 170 mg/m^2. Extrapolating from murine experiments (G.B. Grindey, personal communication; Wedge et al., unpublished results), where the MTD in mice on a folate-supplemented diet was found to be greater than 750 mg/m^2, the MTD in patients may be much higher than the current dose level. Clinical responses, which were observed in early Phase I studies of lometrexol given alone, have not been common in the current study, i.e. only one objective partial response has been observed; however, as the MTD has not been achieved it could be argued that optimal therapeutic conditions have not been defined. One cause for concern is that the administration of folic acid prior to lometrexol and during treatment could potentially supplement the folate requirements of the tumour, and thereby circumvent the activity of lometrexol or, worse still, aid tumour progression [35]. Such a phenomenon would be difficult to examine unequivocally; but the relationship between a patient's plasma folate status and the rate of disease progression might allow this question to be addressed.

Ex. 2031, Laohavinij at 333; Ex. 1075, Schiff Reply ¶¶ 46-50

Prior Art: Laohavinij (Ex. 2031)



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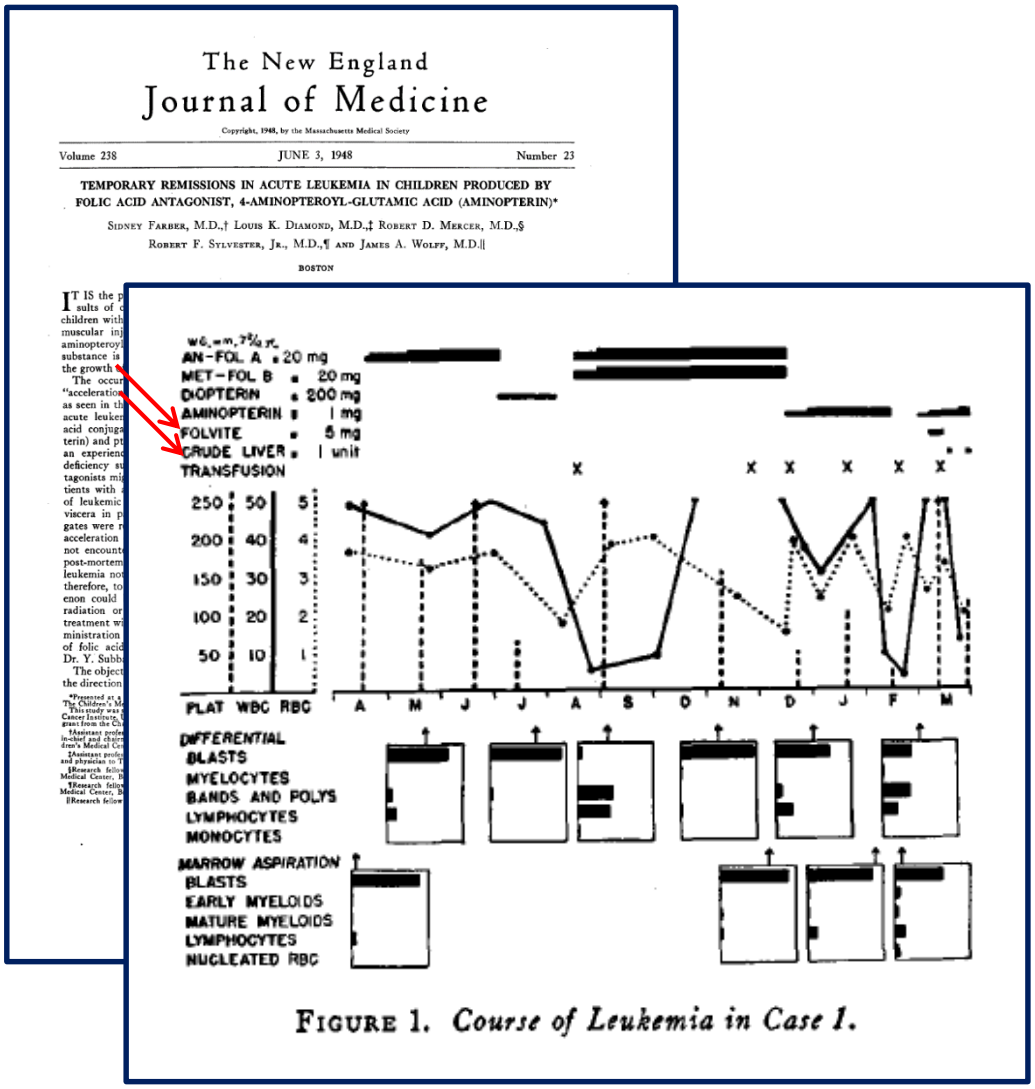
35. Farber S, Diamond LK, Mercer RD et al.: Temporary remissions in acute leukaemia in children produced by folic acid antagonist 4-amethopteroylglutamic acid (aminopterin). New Engl J Med 238:787–793, 1948

Ex. 2031, Laohavinij at 333; Ex. 1075, Schiff Reply ¶¶ 46-50

Prior Art: Farber (Ex. 1009) and Folic Acid and Vitamin B_{12} Supplementation



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TEMPORARY REMISSIONS IN ACUTE LEUKEMIA IN CHILDREN PRODUCED BY FOLIC ACID ANTAGONIST, 4-AMINOPTEROYL-GLUTAMIC ACID (AMINOPTERIN)*

Sidney Farber, M.D.,† Louis K. Diamond, M.D.,‡ Robert D. Mercer, M.D.,§
Robert F. Sylvester, Jr., M.D.,¶ and James A. Wolff, M.D.||
BOSTON

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FIGURE 1. *Course of Leukemia in Case 1.*

On February 3 daily injections of 1 mg. of aminopterin were begun. A bone-marrow biopsy and aspiration revealed 85 per cent blast forms, no megakaryocytes and no erythropoiesis. After 10 days of regular therapy the white-cell count had fallen from 78,000 to 5000, but severe stomatitis made cessation of therapy imperative. Within a week without therapy, the stomatitis had healed completely, and the patient had developed a ravenous appetite. The nutrition gradually improved. By February 21 the liver was no longer palpable, and only the tip of the spleen could be felt. Bone-marrow aspiration and biopsy revealed a slight decrease in blast forms and slight erythropoietic activity. The platelets reached normal levels about 1 month after this course of daily therapy. On March 1 the white-cell count began to rise in spite of daily administration of 0.25 mg. of aminopterin, and by March 6 was 75,000. The spleen again enlarged. The dosage of aminopterin was raised to 1 mg. on March 8, and after about 10 days the white-cell count had fallen to 12,000. Slight stomatitis again appeared, and the dosage of aminopterin was reduced to 0.5 mg. daily, with 1 unit of crude liver extract weekly. The white-cell count has remained at a high-normal level, and the spleen is again slowly receding. The stomatitis is still present but is not progressing and does not interfere with ability to eat.

Ex. 1009, Farber at 788; Ex. 1075, Schiff Reply ¶¶ 96-97; Ex. 2137 Stover Dep. 65-91

Farber Case 1

Prior Art: Farber (Ex. 1009) and Folic Acid and Vitamin B_{12} Supplementation

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ROBERT F. SYLVESTER, JR., M.D.,¶ AND JAMES A. WOLFF, M.D.‖

BOSTON

IT IS the purpose of this paper to record the results of clinical and hematologic studies on 5 children with acute leukemia treated by the intramuscular injection of a synthetic compound, 4-aminopteroylglutamic acid (aminopterin). This substance is an antagonist to folic acid regarding the growth of *Streptococcus faecalis R.*

The occurrence of what he interpreted as an "acceleration phenomenon" in the leukemic process as seen in the marrow and viscera of children with acute leukemia treated by the injection of folic acid conjugates[1] — pteroyltriglutamic acid (teropterin) and pteroyldiglutamic acid (diopterin) — and an experience gained from studies on folic acid deficiency suggested to Farber that folic acid antagonists might be of value in the treatment of patients with acute leukemia.[2] Post-mortem studies of leukemic infiltrates of the bone marrow and viscera in patients treated with folic acid conjugates were regarded by Farber as evidences of an acceleration of the leukemic processes to a degree not encountered in his experience with some 200 post-mortem examinations on children with acute leukemia not so treated. It appeared worth while, therefore, to ascertain if this acceleration phenomenon could be employed to advantage either by radiation or nitrogen mustard therapy after pretreatment with folic acid conjugates or by the administration of antagonists to folic acid.[2] A series of folic acid antagonists was made available by Dr. Y. Subbarow and his colleagues.[3–5]

The objective data sufficient to justify research in the direction of antagonists to folic acid in the treatment of leukemia were obtained from studies on a four-year-old girl with a rapidly progressing acute myelogenous leukemia.[2] Treatment from February 17 to March 24, 1947, with pteroyldiglutamic acid (diopterin), in a dosage of 100 to 300 mg. intramuscularly daily, had no effect upon the hematologic picture. The patient appeared to be moribund. A second bone-marrow biopsy on March 25 verified the diagnosis of myelogenous leukemia. Pteroylaspartic acid, the first antagonist to folic acid to be employed in our studies, was given intramuscularly from March 28 to April 4 in amounts of 40 mg. daily without altering the clinical course. Post-mortem examination on April 4 revealed a markedly hypoplastic bone marrow, with a few immature cells. A change of this magnitude in such a short time has not been encountered in the marrow of leukemic children in our experience.

This observation was followed by clinical, laboratory, and post-mortem studies** on a group of 14 children with acute leukemia treated with pteroylaspartic acid and on 7 treated with methylpteroic acid. The details of these observations will be reported separately.

Sufficient encouragement was obtained from these observations to justify further studies on the effect of more powerful antagonists to folic acid on the course of acute leukemia in children. Since November, 1947, when a sufficiently pure substance became available, to the time of this writing (April 15, 1948) we have made studies on 16 children with acute leukemia to whom the most powerful folic antagonist we have yet encountered, 4-aminopteroylglutamic acid (aminopterin††) was administered by intramuscular injection. Many of these children were moribund at the onset of therapy. Of 16 infants and children with acute leukemia treated with aminopterin 10 showed clinical, hematologic and pathological evidences of improvement of important

*Presented at a meeting of the Division of Laboratories and Research, The Children's Medical Center, Boston, April 8, 1948.
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††This compound was first synthesized by the Calco Chemical Division of the American Cyanamid Company.

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**Sandoz Inc.
Exhibit 1009-0001**

The patient was treated with pteroylaspartic acid beginning on April 16 in doses of 20 to 60 mg. daily while in the hospital, in a convalescent home, where he remained until May 20, and at home, where the injections were given by the family physician. During that time he was active and fairly well, although the white-cell count remained high and the red-cell count and hemoglobin fell slowly.

On July 1 diopterin, in a dosage of 200 mg. by mouth daily, was begun. This therapy was continued for about 1 month, during which the patient steadily became more ill. The liver and spleen enlarged, and he became very anemic. The blast forms in the peripheral blood rose to 94 per cent. Joint pain and fever recurred, and by August 13 he was critically ill, with a temperature reaching 106°F. He was readmitted to the hospital and received several transfusions. Pteroylaspartic acid and methylpteroic acid, in doses of 40 mg. each, were given intramuscularly daily. The patient was discharged after about 2 weeks, and pteroylaspartic acid and methylpteroic acid, in doses of 20 mg. each, were continued in the Tumor Therapy Office. A period of remission ensued, during which the red-cell count and platelets returned to normal levels, the liver and spleen receded in size and the nutrition improved remarkably. He returned to school part time in October and was in quite good condition. The white-cell count had risen to high levels, however, and in November general deterioration began. The liver and spleen enlarged, and he became so anemic that transfusion was necessary by November 24. Only temporary benefit resulted and transfusions were required at about 3-week intervals.

Ex. 1009, Farber at 788; Schiff Reply ¶96

Prior Art: Farber (Ex. 1009) and Vitamin B_{12} Supplementation

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††This compound was first synthesized by the Calco Chemical Division of the American Cyanamid Company.

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Exhibit 1009-0001

Farber Case 2

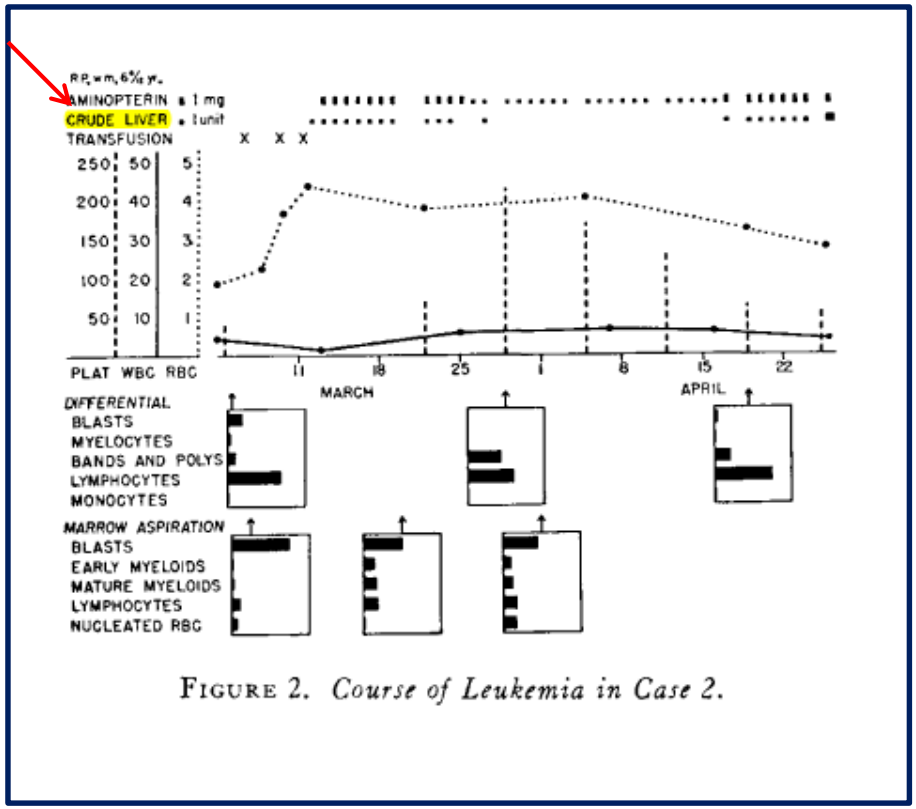
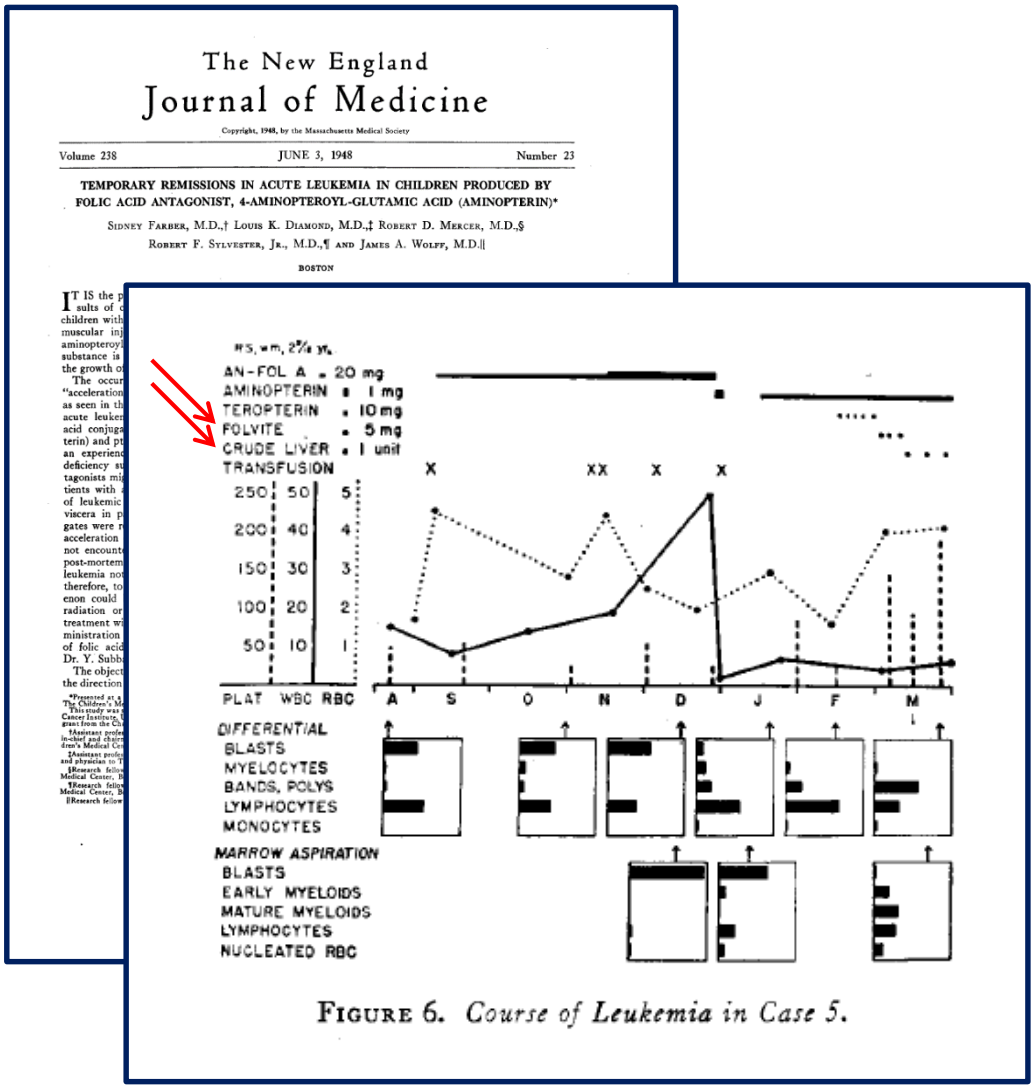


FIGURE 2. *Course of Leukemia in Case 2.*

Ex. 1009, Farber at 789; Ex. 1075, Schiff Reply ¶96; Stover Reply ¶17; Stover Dep. 65-91

Prior Art: Farber (Ex. 1009) and Folic Acid and Vitamin B_{12} Supplementation



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ROBERT F. SYLVESTER, JR., M.D.,¶ AND JAMES A. WOLFF, M.D.||
BOSTON

AN-FOL A = 20 mg
AMINOPTERIN = 1 mg
TEROPTERIN = 10 mg
FOLVITE = 5 mg
CRUDE LIVER = 1 unit
TRANSFUSION

PLAT WBC RBC

DIFFERENTIAL
BLASTS
MYELOCYTES
BANDS, POLYS
LYMPHOCYTES
MONOCYTES

MARROW ASPIRATION
BLASTS
EARLY MYELOIDS
MATURE MYELOIDS
LYMPHOCYTES
NUCLEATED RBC

FIGURE 6. *Course of Leukemia in Case 5.*

Under treatment with aminopterin the white-cell count tended to return to a normal level. This occurred in patients in whom the count was initially high and also in those in whom there was marked leukopenia at the onset of the therapy. The percentage of immature cells fell, and the blast forms decreased markedly and in some cases disappeared from the peripheral blood. The relative percentages of mature leukocytes tended to approach normal values in the peripheral blood. The peripheral-blood changes included improvement approaching the normal in the value of hemoglobin, red-cell count and platelets. Studies of the bone marrow showed changes that varied from a decrease in number to a disappearance of the leukemic cells and variation from hypoplasia to almost normal pattern. Toxic effects included stomatitis, with early ulceration. In an attempt to prevent this complication crude liver extract was employed, as were folic acid and folic acid conjugates.

Ex. 1009, Farber at 792; Ex. 1075, Schiff Reply ¶96; Ex. 1091, Stover Reply ¶17; Ex. 2137, Stover Dep. 65-91

Farber Case 5

Prior Art: Farber (Ex. 1009) and Folic Acid and Vitamin B_{12} Supplementation

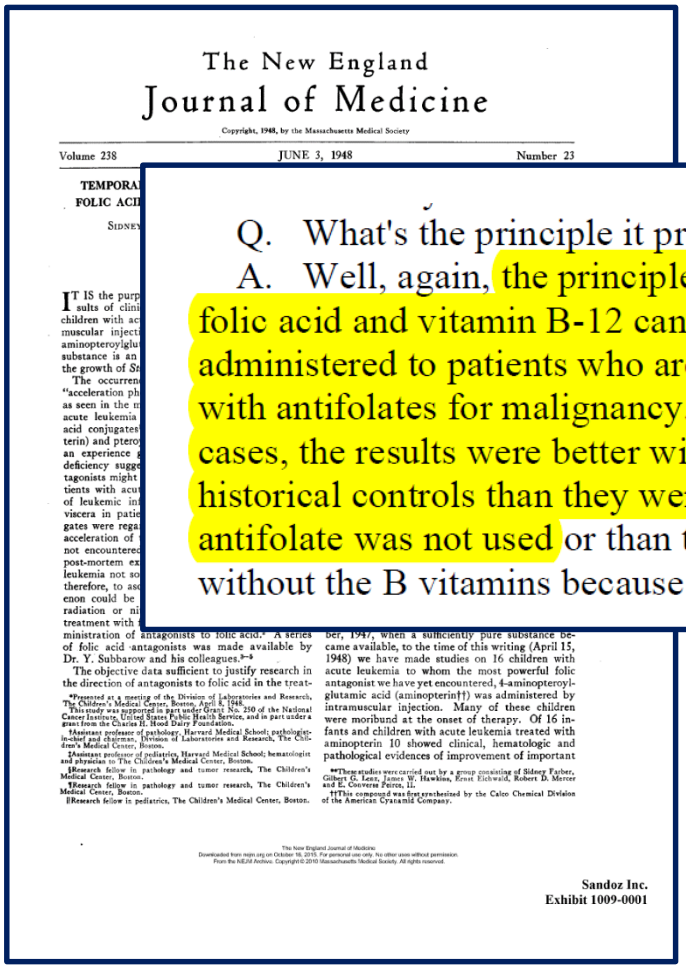

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Exhibit 1009-0001

Q. What's the principle it proves?
A. Well, again, the principle is that folic acid and vitamin B-12 can be administered to patients who are also treated with antifolates for malignancy. And in some cases, the results were better with historical controls than they were if the antifolate was not used or than they were without the B vitamins because --

Ex. 2136, Schiff Dep. ¶45:10-46:25; Paper 70, 4; Ex. 1075, Schiff reply ¶96

continued use of the drug impossible." (Ex. 1009, Farber at 792.) Thus, Dr. Farber realized early on that an antifolate, such as aminopterin, was highly toxic and folic acid regimens could ameliorate these toxicities. The doses of folic acid Dr. Farber used to treat these toxicities (5 mg folvite, which is folic acid, and 1 unit of crude liver extract) exceeded the recommended daily allowance for folic acid, a point on which Dr. Zeisel agrees. (Ex. 1086, Zeisel Dep 146:21-24.) In addition, crude liver extracts have long been known to contain vitamin B_{12} useful for treating pernicious anemia. (Ex. 1109, Cuthbertson at 708.)

Ex. 1091, Stover Reply ¶17

Prior Art: Farber (Ex. 1009) and Folic Acid and Vitamin B_{12} Supplementation

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TEMPORARY REMISSIONS IN ACUTE LEUKEMIA IN CHILDREN PR
FOLIC ACID ANTAGONIST, 4-AMINOPTEROYL-GLUTAMIC ACID (AM

SIDNEY FARBER, M.D.,† LOUIS K. DIAMOND, M.D.,‡ ROBERT D. MERCE
ROBERT F. SYLVESTER, JR., M.D.,¶ AND JAMES A. WOLFF, M.D
BOSTON

IT IS the purpose of this paper to record the results of clinical and hematologic studies on 5 children with acute leukemia treated by the intramuscular injection of a synthetic compound, 4-aminopteroylglutamic acid (aminopterin). This substance is an antagonist to folic acid regarding the growth of *Streptococcus faecalis R.*

The occurrence of what he interpreted as an "acceleration phenomenon" in the leukemic process as seen in the marrow and viscera of children with acute leukemia treated by the injection of folic acid conjugates[1] — pteroyltriglutamic acid (teropterin) and pteroyldiglutamic acid (diopterin) — and an experience gained from studies on folic acid deficiency suggested to Farber that folic acid antagonists might be of value in the treatment of patients with acute leukemia.[2] Post-mortem studies of leukemic infiltrates of the bone marrow and viscera in patients treated with folic acid conjugates were regarded by Farber as evidences of an acceleration of the leukemic processes to a degree not encountered in his experience with some 200 post-mortem examinations on children with acute leukemia not so treated. It appeared worth while, therefore, to ascertain if this acceleration phenomenon could be employed to advantage either by radiation or nitrogen mustard therapy after pretreatment with folic acid conjugates or by the administration of antagonists to folic acid.[2] A series of folic acid antagonists was made available by Dr. Y. Subbarow and his colleagues.[3–5]

The objective data sufficient to justify research in the direction of antagonists to folic acid in the treatment of leukemia were obtaine
four-year-old girl with a rapidl
myelogenous leukemia.[2] Treat
ary 17 to March 24, 1947, wit
acid (diopterin), in a dosage of
muscularly daily, had no effect u
picture. The patient appeared
second bone-marrow biopsy on
the diagnosis of myelogenous l
spartic acid, the first antagonis
employed in our studies, was gi
from March 28 to April 4 in
daily without altering the cli
mortem examination on April 4
hypoplastic bone marrow, wit
cells. A change of this magnit
time has not been encountere
leukemic children in our experie

This observation was followe
tory, and post-mortem studies
children with acute leukemia t
aspartic acid and on 7 treated
acid. The details of these obse
ported separately.

Sufficient encouragement was
observations to justify further
of more powerful antagonists
course of acute leukemia in chil
ber, 1947, when a sufficiently
came available, to the time of t
1948) we have made studies
acute leukemia to whom the
antagonist we have yet encounte
glutamic acid (aminopterin††)
intramuscular injection. Many
were moribund at the onset of
fants and children with acute le
aminopterin 10 showed clinical, hematologic and pathological evidences of improvement of important

*Presented at a meeting of the Division of Laboratories and Research, The Children's Medical Center, Boston, April 8, 1948.
This study was supported in part under Grant No. 250 of the National Cancer Institute, United States Public Health Service, and in part under a grant from the Charles H. Hood Dairy Foundation.

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¶Research fellow in pathology and tumor research, The Children's Medical Center, Boston.
‖Research fellow in pediatrics, The Children's Medical Center, Boston.

**These studies were carried out by a group consisting of Sidney Farber, Gilbert G. Lenz, James W. Hawkins, Ernst Eichwald, Robert D. Mercer and E. Converse Peirce, II.

††This compound was first synthesized by the Calco Chemical Division of the American Cyanamid Company.

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Exhibit 1009-0001

12 And that's -- but that's not a connection
13 anyone expressly made in the literature; that's just
14 what you're bringing to it with your knowledge of
15 the pathways?
16 A. There is an extensive literature on the
17 interaction between vitamin B12 and folate that --
18 that goes back to this time period up to 1999.
19 So, in my opinion, and I would strongly
20 argue in the opinion of anyone with knowledge in
21 this area, would certainly view a treatment with a
22 rat liver extract -- I mean a liver -- rat liver --
23 with liver extract as addressing nutritional
24 deficiencies associated with the entire pathway.
25 I mean, I view that as -- as something that
2 would be common knowledge. There is a strong
3 literature relating to the interactions of folate
4 and vitamin B12.

Ex. 2137, Stover Dep. 90:12-91:4

5 Q. And are you aware of any literature from
6 the time of Farber forward to 1999 that attached any
7 particular significance to the fact that vitamin B12
8 was a component of Farber's liver extract?
9 A. Anyone with knowledge, fundamental
10 knowledge of human physiology, of human
11 biochemistry, of nutrition would implicitly know
12 that liver extract has vitamin B12 in it.

Ex. 2137, Stover Dep. 82:5-12

Prior Art: Worzalla (Ex. 1013)

ANTICANCER RESEARCH *18*: 3235-3240 (1998)

Role of Folic Acid in Modulating the Toxicity and Efficacy of the Multitargeted Antifolate, LY231514

JOHN F. WORZALLA, CHUAN SHIH and RICHARD M. SCHULTZ

Cancer Research Division, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285, U.S.A.

Abstract. *We studied the effects of folic acid on modulating the toxicity and antitumor efficacy of LY231514. Using several human tumor cell lines adapted to growth in low folate medium, folic acid was shown to be 100- to 1000-fold less active than folinic acid at protecting cells from LY231514-induced cytotoxicity. The lethality of LY231514 was compared in mice maintained on standard diet or low folate diet. The LD50 occurred at 60- and 250-fold lower doses of LY231514 in DBA/2 and CD1 nu/nu mice, respectively, maintained on low folate diet compared to standard diet. The L5178Y/TK-/HX- murine lymphoma was much more sensitive to the antitumor action of LY231514 compared to wild type L5178Y-S tumors. For mice on low folate diet, LY231514 at 0.3 and 1 mg/kg (qd x 10, i.p.) produced 100% inhibition of L5178Y/TK-/HX- lymphoma growth, and significant lethality occurred at ≥ 3 mg/kg. For mice on standard diet, LY231514 produced >95% inhibition of tumor growth at 30 to 300 mg/kg, but all mice died at 800 mg/kg. Folic acid supplementation was demonstrated to preserve the antitumor activity of LY231514 while reducing toxicity. The combination of folic acid with LY231514 may provide a mechanism for enhanced clinical antitumor selectivity.*

LY231514 is a structurally novel antifolate antimetabolite that possesses the unique 6-5-fused pyrrolo[2,3-d]pyrimidine nucleus (1) instead of the more common 6-6-fused pteridine or quinazoline ring structure. The primary mode of antitumor activity for LY231514 has previously been ascribed to inhibition of thymidylate synthase (TS) (1, 2). However, several lines of evidence suggest that multiple enzyme-inhibitory mechanisms are involved in cytotoxicity, hence the acronym MTA (multitargeted antifolate): 1) the reversal pattern for MTA in human leukemia and colon carcinoma cell lines demonstrates that although TS may be a major site of action for LY231514 at concentrations near the IC50, higher concentrations can lead to inhibition of dihydrofolate reductase (DHFR) and/or other enzymes along the purine de novo pathway (3); 2) MTA is an excellent substrate for folylpolyglutamate synthetase, and the K_i values of the pentaglutamate of LY231514 are 1.3, 7.2, and 65 nM for inhibition against TS, DHFR and glycinamide ribonucleotide formyltransferase (GARFT), respectively (3); 3) intracellular concentrations of LY231514 and its polyglutamates can exceed 40 μM in CCRF-CEM cells when ^{3}H-labeled LY231514 was used (R.M. Schultz, unpublished observation); and 4) early clinical studies demonstrated that patients who had previously failed to respond to ZD1694 and 5-fluorouracil/leucovorin treatment responded to LY231514 (4; DA Rinaldi, personal communication).

Several animal studies have indicated that folic acid supplementation in combination with antifolate cancer therapy can prevent delayed toxicity and enhance the therapeutic potential of the GARFT inhibitor lometrexol (5, 6) and the TS inhibitor 1843U89 (7). Unexpected delayed cumulative toxicity was observed in phase I studies with lometrexol, including thrombocytopenia, anemia, and mucositis (8). Additional clinical studies demonstrated the protective effects of folic acid against lometrexol toxicity in humans (9). Morgan and coworkers (10) concluded that a daily supplement of 1 mg of folic acid during low-dose methotrexate therapy in patients with rheumatoid arthritis was useful in lessening toxicity without altering efficacy. In the present communication, we investigated the effects of folic acid on the antitumor activity and lethality of LY231514 in mice.

Materials and Methods

Reagents. Folic acid, folinic acid (leucovorin), and 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The disodium salt of LY231514 was synthesized at Eli Lilly and Co. (1).

Cell lines. Human CCRF-CEM leukemia cells were obtained from St. Jude Children's Research Hospital (Memphis, TN, USA). Human IGROV1 ovarian carcinoma cells were generously supplied by Dr.

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Key Words: LY231514, antitumor activity, antifolate, folic acid.

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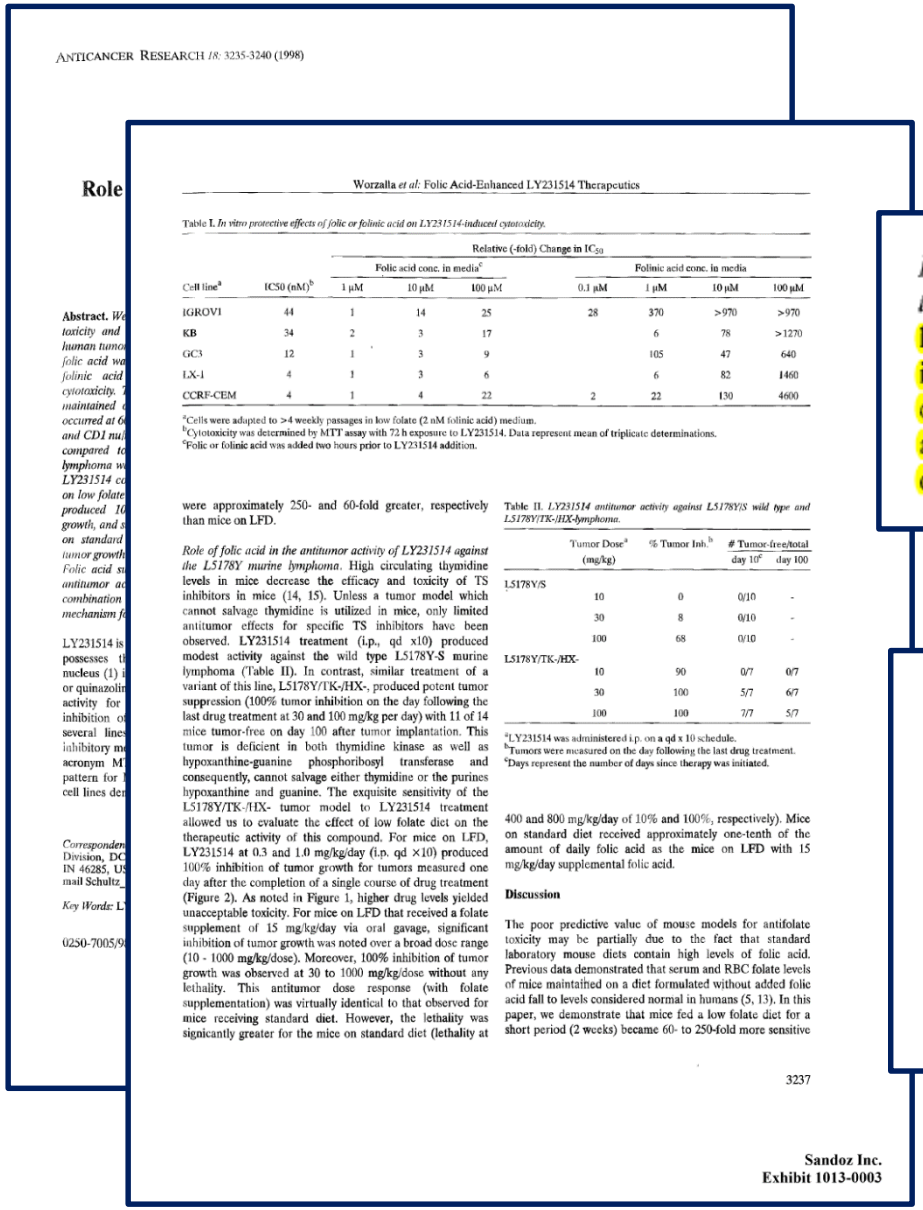
Exhibit 1013-0001

Abstract. *We studied the effects of folic acid on modulating the toxicity and antitumor efficacy of LY231514. Using several human tumor cell lines adapted to growth in low folate medium, folic acid was shown to be 100- to 1000-fold less active than folinic acid at protecting cells from LY231514-induced cytotoxicity. The lethality of LY231514 was compared in mice maintained on standard diet or low folate diet. The LD50 occurred at 60- and 250-fold lower doses of LY231514 in DBA/2 and CD1 nu/nu mice, respectively, maintained on low folate diet compared to standard diet. The L5178Y/TK-/HX- murine lymphoma was much more sensitive to the antitumor action of LY231514 compared to wild type L5178Y-S tumors. For mice on low folate diet, LY231514 at 0.3 and 1 mg/kg (qd x 10, i.p.) produced 100% inhibition of L5178Y/TK-/HX- lymphoma growth, and significant lethality occurred at ≥ 3 mg/kg. For mice on standard diet, LY231514 produced >95% inhibition of tumor growth at 30 to 300 mg/kg, but all mice died at 800 mg/kg. Folic acid supplementation was demonstrated to preserve the antitumor activity of LY231514 while reducing toxicity. The combination of folic acid with LY231514 may provide a mechanism for enhanced clinical antitumor selectivity.*

Ex. 1013, p. 3235; Ex. 1004, Schiff ¶¶ 57-62;
Ex. 1075, Schiff Reply, ¶¶ 55-60.

Prior Art: Worzalla (Ex. 1013)



ANTICANCER RESEARCH *18*: 3235-3240 (1998)

Role

Worzalla *et al*: Folic Acid-Enhanced LY231514 Therapeutics

Table I. *In vitro protective effects of folic or folinic acid on LY231514-induced cytotoxicity.*

Cell line[a]	IC50 (nM)[b]	Relative (-fold) Change in IC_{50}						
		Folic acid conc. in media[c]			Folinic acid conc. in media			
		1 μM	10 μM	100 μM	0.1 μM	1 μM	10 μM	100 μM
IGROV1	44	1	14	25	28	370	>970	>970
KB	34	2	3	17		6	78	>1270
GC3	12	1	3	9		105	47	640
LX-1	4	1	3	6		6	82	1460
CCRF-CEM	4	1	4	22	2	22	130	4600

[a]Cells were adapted to >4 weekly passages in low folate (2 nM folinic acid) medium.
[b]Cytotoxicity was determined by MTT assay with 72 h exposure to LY231514. Data represent mean of triplicate determinations.
[c]Folic or folinic acid was added two hours prior to LY231514 addition.

were approximately 250- and 60-fold greater, respectively than mice on LFD.

Role of folic acid in the antitumor activity of LY231514 against the L5178Y murine lymphoma. High circulating thymidine levels in mice decrease the efficacy and toxicity of TS inhibitors in mice (14, 15). Unless a tumor model which cannot salvage thymidine is utilized in mice, only limited antitumor effects for specific TS inhibitors have been observed. LY231514 treatment (i.p., qd x10) produced modest activity against the wild type L5178Y-S murine lymphoma (Table II). In contrast, similar treatment of a variant of this line, L5178Y/TK-/HX-, produced potent tumor suppression (100% tumor inhibition on the day following the last drug treatment at 30 and 100 mg/kg per day) with 11 of 14 mice tumor-free on day 100 after tumor implantation. This tumor is deficient in both thymidine kinase as well as hypoxanthine-guanine phosphoribosyl transferase and consequently, cannot salvage either thymidine or the purines hypoxanthine and guanine. The exquisite sensitivity of the L5178Y/TK-/HX- tumor model to LY231514 treatment allowed us to evaluate the effect of low folate diet on the therapeutic activity of this compound. For mice on LFD, LY231514 at 0.3 and 1.0 mg/kg/day (i.p. qd ×10) produced 100% inhibition of tumor growth for tumors measured one day after the completion of a single course of drug treatment (Figure 2). As noted in Figure 1, higher drug levels yielded unacceptable toxicity. For mice on LFD that received a folate supplement of 15 mg/kg/day via oral gavage, significant inhibition of tumor growth was noted over a broad dose range (10 - 1000 mg/kg/dose). Moreover, 100% inhibition of tumor growth was observed at 30 to 1000 mg/kg/dose without any lethality. This antitumor dose response (with folate supplementation) was virtually identical to that observed for mice receiving standard diet. However, the lethality was significantly greater for the mice on standard diet (lethality at

Table II. *LY231514 antitumor activity against L5178Y/S wild type and L5178Y/TK-/HX-lymphoma.*

	Tumor Dose[a] (mg/kg)	% Tumor Inh.[b]	# Tumor-free/total	
			day 10[c]	day 100
L5178Y/S				
	10	0	0/10	-
	30	8	0/10	-
	100	68	0/10	-
L5178Y/TK-/HX-				
	10	90	0/7	0/7
	30	100	5/7	6/7
	100	100	7/7	5/7

[a]LY231514 was administered i.p. on a qd x 10 schedule.
[b]Tumors were measured on the day following the last drug treatment.
[c]Days represent the number of days since therapy was initiated.

400 and 800 mg/kg/day of 10% and 100%, respectively). Mice on standard diet received approximately one-tenth of the amount of daily folic acid as the mice on LFD with 15 mg/kg/day supplemental folic acid.

Discussion

The poor predictive value of mouse models for antifolate toxicity may be partially due to the fact that standard laboratory mouse diets contain high levels of folic acid. Previous data demonstrated that serum and RBC folate levels of mice maintained on a diet formulated without added folic acid fall to levels considered normal in humans (5, 13). In this paper, we demonstrate that mice fed a low folate diet for a short period (2 weeks) became 60- to 250-fold more sensitive

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Exhibit 1013-0003

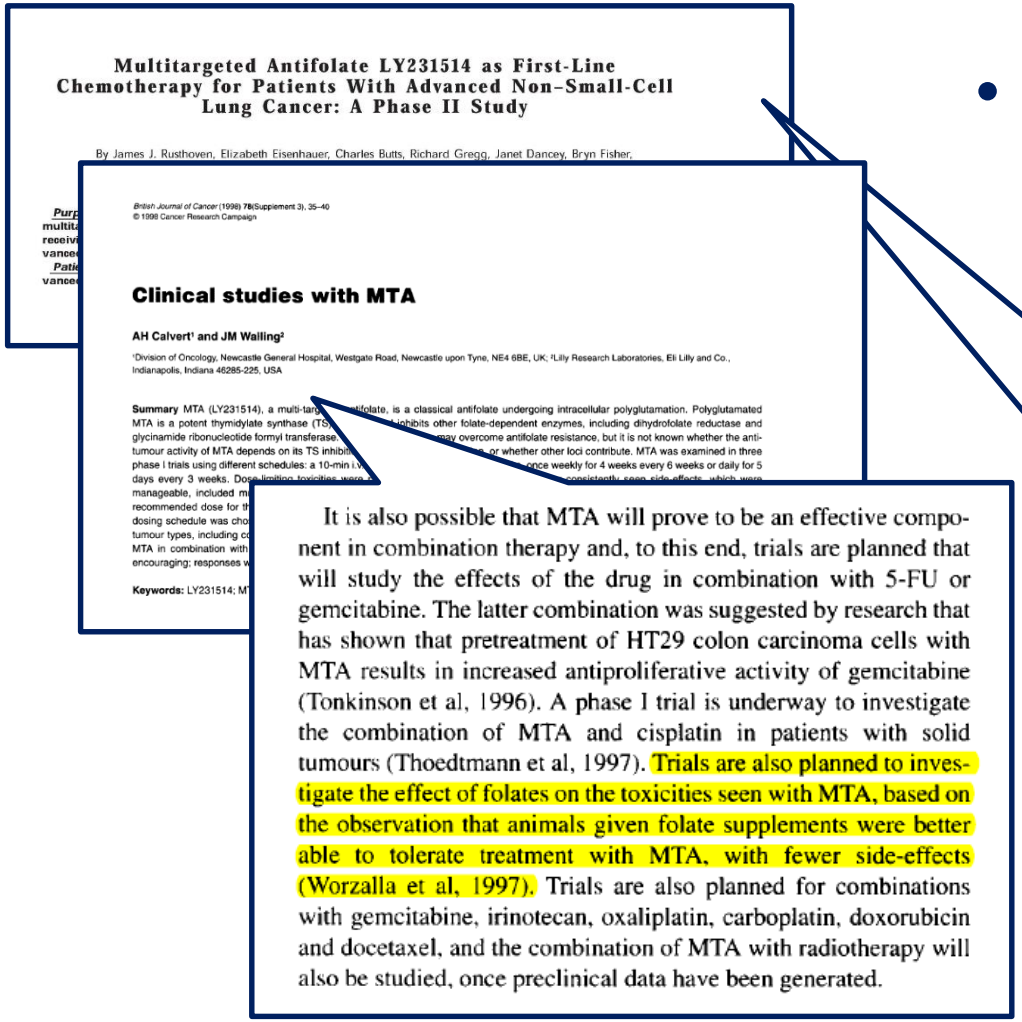
Role of folic acid in the antitumor activity of LY231514 against the L5178Y murine lymphoma. High circulating thymidine levels in mice decrease the efficacy and toxicity of TS inhibitors in mice (14, 15). Unless a tumor model which cannot salvage thymidine is utilized in mice, only limited antitumor effects for specific TS inhibitors have been observed. LY231514 treatment (i.p., qd x10) produced

Ex. 1013, p. 3237; Ex. 1004, Schiff ¶¶ 58-59;
Ex. 1075, Schiff Reply, ¶¶55-56.

The poor predictive value of mouse models for antifolate toxicity may be partially due to the fact that standard laboratory mouse diets contain high levels of folic acid. Previous data demonstrated that serum and RBC folate levels of mice maintained on a diet formulated without added folic acid fall to levels considered normal in humans (5, 13). In this paper, we demonstrate that mice fed a low folate diet for a short period (2 weeks) became 60- to 250-fold more sensitive to the lethality of LY231514 than observed in mice fed standard laboratory diet (Figure 1). The antifolate GARFT

Ex. 1013, p. 3237-38; Ex. 1004, Schiff ¶¶ 58-59;
Ex. 1075, Schiff Reply, ¶¶55-56.

Prior Art: Worzalla (Ex. 1013)



Multitargeted Antifolate LY231514 as First-Line Chemotherapy for Patients With Advanced Non–Small-Cell Lung Cancer: A Phase II Study

By James J. Rusthoven, Elizabeth Eisenhauer, Charles Butts, Richard Gregg, Janet Dancey, Bryn Fisher,

British Journal of Cancer (1998) 78(Supplement 3), 35–40
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Clinical studies with MTA

AH Calvert[1] and JM Walling[2]

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It is also possible that MTA will prove to be an effective component in combination therapy and, to this end, trials are planned that will study the effects of the drug in combination with 5-FU or gemcitabine. The latter combination was suggested by research that has shown that pretreatment of HT29 colon carcinoma cells with MTA results in increased antiproliferative activity of gemcitabine (Tonkinson et al, 1996). A phase I trial is underway to investigate the combination of MTA and cisplatin in patients with solid tumours (Thoedtmann et al, 1997). Trials are also planned to investigate the effect of folates on the toxicities seen with MTA, based on the observation that animals given folate supplements were better able to tolerate treatment with MTA, with fewer side-effects (Worzalla et al, 1997). Trials are also planned for combinations with gemcitabine, irinotecan, oxaliplatin, carboplatin, doxorubicin and docetaxel, and the combination of MTA with radiotherapy will also be studied, once preclinical data have been generated.

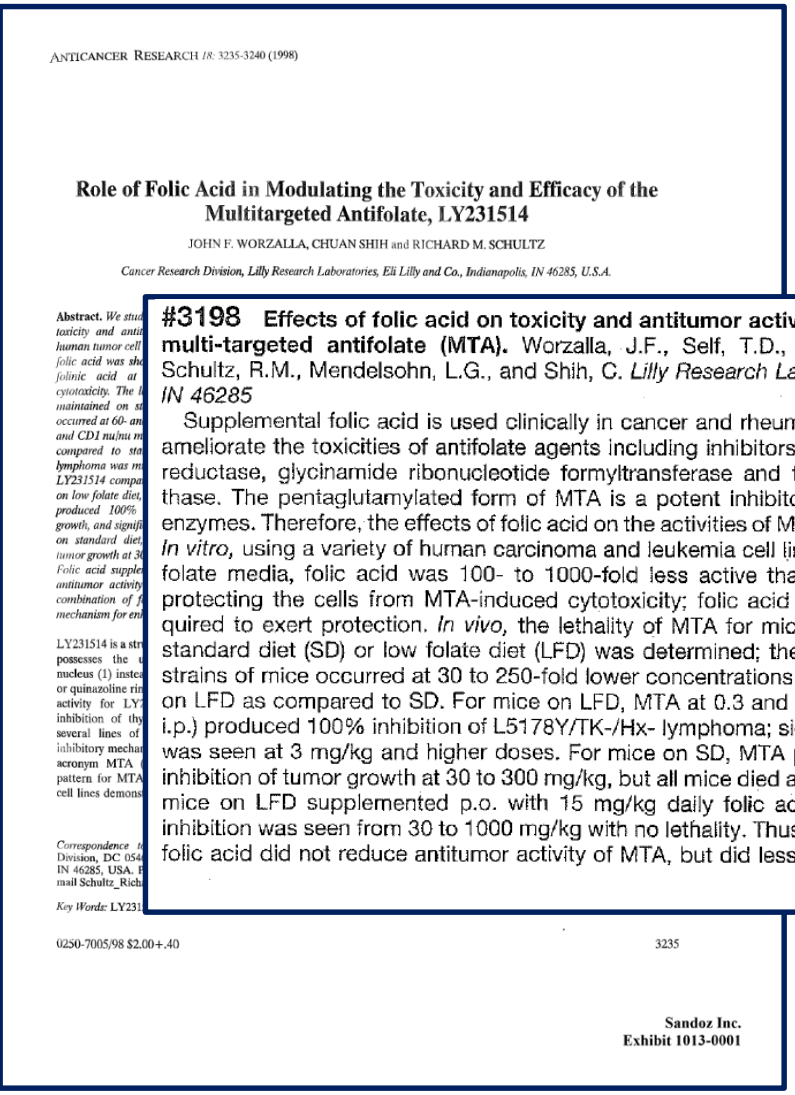
Ex. 1047, Calvert & Walling, at 39 (citing Ex. 1101, Worzalla at abstract); Ex. 1074, Chabner Dep. 226:15-227:12

- Worzalla cited by others

MTA has demonstrated activity in a wide range of tumor types. The drug is highly active against CCRF-CEM human leukemia cells in vitro; the activity is partially reversible with the addition of thymidine.[12-14] The 50% inhibitory concentration in CCRF-CEM cells was 7 ng/mL.[13] It is also cytotoxic in human tumor colony-forming unit assays against human colon, renal, small-cell lung and non–small-cell lung cancers, hepatomas, and carcinoid tumors.[16] MTA can inhibit tumor growth in mice transplanted with human colon xenografts resistant to methotrexate.[17] In beagle dogs treated with a weekly and/or single-dose intravenous (IV) schedule, major toxicities included anorexia, emesis, diarrhea, mucositis, weight loss, neutropenia, lymphopenia, and mild anemia. Plasma concentrations increased linearly with increasing doses, with the terminal half-life occurring at about 2.3 hours.[18] Early studies have suggested that dietary supplementation with folic acid may improve the therapeutic index by reducing toxicity in mice.

Ex. 1052, Rusthoven, at 1195.

Prior Art: Citations to Preclinical Worzalla Studies (Ex. 1013 and Ex. 1101)



ANTICANCER RESEARCH *18*: 3235-3240 (1998)

Role of Folic Acid in Modulating the Toxicity and Efficacy of the Multitargeted Antifolate, LY231514

JOHN F. WORZALLA, CHUAN SHIH and RICHARD M. SCHULTZ

Cancer Research Division, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285, U.S.A.

#3198 Effects of folic acid on toxicity and antitumor activity of LY231514 multi-targeted antifolate (MTA). Worzalla, J.F., Self, T.D., Theobald, K.S., Schultz, R.M., Mendelsohn, L.G., and Shih, C. *Lilly Research Labs, Indianapolis, IN 46285*

Supplemental folic acid is used clinically in cancer and rheumatoid arthritis to ameliorate the toxicities of antifolate agents including inhibitors of dihydrofolate reductase, glycinamide ribonucleotide formyltransferase and thymidylate synthase. The pentaglutamylated form of MTA is a potent inhibitor of these three enzymes. Therefore, the effects of folic acid on the activities of MTA were studied. *In vitro,* using a variety of human carcinoma and leukemia cell lines grown in low folate media, folic acid was 100- to 1000-fold less active than folinic acid at protecting the cells from MTA-induced cytotoxicity; folic acid >1 μM was required to exert protection. *In vivo,* the lethality of MTA for mice maintained on standard diet (SD) or low folate diet (LFD) was determined; the LD_{50} in several strains of mice occurred at 30 to 250-fold lower concentrations of MTA for mice on LFD as compared to SD. For mice on LFD, MTA at 0.3 and 1 mg/kg (qdx10, i.p.) produced 100% inhibition of L5178Y/TK-/Hx- lymphoma; significant lethality was seen at 3 mg/kg and higher doses. For mice on SD, MTA produced >95% inhibition of tumor growth at 30 to 300 mg/kg, but all mice died at 800 mg/kg. For mice on LFD supplemented p.o. with 15 mg/kg daily folic acid, 100% tumor inhibition was seen from 30 to 1000 mg/kg with no lethality. Thus, addition of oral folic acid did not reduce antitumor activity of MTA, but did lessen toxicity.

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Exhibit 1013-0001

icity while maintaining efficacy. More recently, Worzalla et al. reported that, in mice that were given folic acid with pemetrexed, the incidence and severity of adverse events were lowered dramatically, whereas the antitumor activity was preserved.[30] Concurrent

Ex. 1079, Paz Ares, 2059.

pemetrexed plus cisplatin arm. Preclinical data also became available that suggested that folate supplementation might reduce toxicity without reducing efficacy.[23] These data led to the institution of folic acid and B_{12} supplementation in all ongoing trials, including the mesothelioma trial. As

Ex. 1078, Bunn, 20-21.

safely. Much of the basis for use of FA as a pharmacologic modulating agent is associated with studies conducted in animal models.[1,5-7] However,

Ex. 1103, Priest, 38.

Ex. 1075, Schiff Reply ¶55 (collection of references).

Prior Art: Worzalla (Ex. 1013)

ANTICANCER RESEARCH *18*: 3235-3240 (1998)

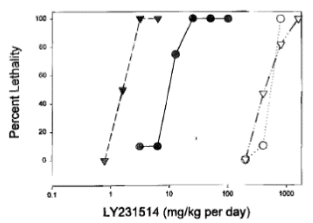

Figure 1. *The toxicity of LY231514 in mice is increased by a folate-deficient diet. DBA/2 and CDI nu/nu mice were fed either a standard laboratory diet (○ and ▽, respectively) or a folate-deficient diet for 2 weeks prior to the first dose of LY231514 (● and ▼, respectively) and for the duration of the study. Groups of mice (> 10 animals/group) on each diet were given 10 daily doses of LY231514 i.p. at the indicated doses. The data present the percent lethality within 3 weeks after the last dose of LY231514.*

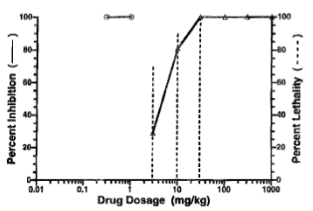

Figure 2. *Antitumor activity of LY231514 therapy (i.p., qd ×10) against L5178Y/TK- /HX- lymphoma for mice on low folate diet with no folate supplementation (○) and for mice on low folate diet that received 15 mg/kg/day daily folate supplementation (△). Vertical dashed lines represent percent lethality in mice on low folate diet with no folate supplementation. No lethality was observed in mice that received folate supplementation.*

to the lethality of LY231514 than observed in mice fed standard laboratory diet (Figure 1). The antifolate GARFT inhibitor, lometrexol has previously been shown to accumulate in the livers of folate-deficient mice, and this accumulation was diminished by the administration of folic acid to these animals (16). These investigators hypothesized that the substantial and unexpected toxicity of lometrexol in humans not given concurrent folic acid and in folate-deficient mice is due to the sequestration of drug in hepatic tissue, with the subsequent slow release of drug to the circulation at toxicologically relevant concentrations. The mechanism for this accumulation of lometrexol in liver probably involves metabolism to polyglutamate forms by the enzyme folylpoly-γ-glutamate synthetase (FPGS). In this regard, Mendelsohn and coworkers (6) demonstrated that liver produced the greatest response in elevated FPGS to low dietary folate of all tissues tested. A similar mechanism probably exists for the potentiation of LY231514 toxicity by folate-deficient diet, since this compound is an extremely efficient substrate for mouse liver FPGS (1). In addition, LY231514 requires polyglutamation for cytotoxic potency (3).

The uptake of natural reduced folate compounds and folate analogues into cells appears to involve membrane protein receptors of two different classes: a reduced folate/methotrexate carrier (RFC), which binds reduced folate in the micromolar range, and a high-affinity folate binding protein (mFBP), which preferentially binds to oxidized folate and other analogs with an affinity <1 nM (17). Studies using a panel of ZR-75-1 human breast sublines with differing transport properties have demonstrated a predominant role for the RFC in intracellular transport of LY231514 (3). Similarly, we now report that folic acid only weakly modulates the cytotoxic activity of LY231514 for various human leukemia and carcinoma cells adapted to low folate conditions (Table I). Some of these cells (KB and IGROV1) have previously been demonstrated to possess elevated levels of mFBP (18), further suggesting a minor role for mFBP in LY231514 transport.

LY231514 produced potent antitumor activity against the L5178Y/TK-/HX- lymphoma at 100-fold lower dose levels (0.3 and 1 mg/kg/day, Figure 2) in LFD mice relative to 30 and 100 mg/kg (Table II) in mice on standard diet. It is interesting to note that the LD_{50} was reduced 3000-fold for lometrexol in LFD animals, and antitumor activity could not be demonstrated even at low dose levels (5). In contrast, the shift in both LD_{50} and antitumor activity for mice on LFD compared to standard diet were of a similar magnitude (approximately 100-fold) for LY231514. However, LFD animals with high levels of folate supplementation demonstrated decreased lethality to LY231514 compared to conventional diet animals, suggesting that folate intake can be manipulated to achieve greater therapeutic effects. Oral folic acid dramatically decreased the toxicity of LY231514 and preserved antitumor activity (albeit at higher dose levels) in these mice (Figure 2).

Previous studies have demonstrated that the multitargeted antifolate, LY231514 has a unique biochemical and pharmacological profile. Exciting antitumor activity has been observed in phase I and II clinical trials, including responses in colon, breast, non-small cell lung and pancreatic cancers. More advanced and extensive clinical trials of LY231514 are currently in progress. The combination of folic acid with

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Exhibit 1013-0004

Figure 2. *Antitumor activity of LY231514 therapy (i.p., qd ×10) against L5178Y/TK- /HX- lymphoma for mice on low folate diet with no folate supplementation (○) and for mice on low folate diet that received 15 mg/kg/day daily folate supplementation (△). Vertical dashed lines represent percent lethality in mice on low folate diet with no folate supplementation. No lethality was observed in mice that received folate supplementation.*

Ex. 1013, p. 3238; Ex. 1004, Schiff ¶¶ 57-62; Ex. 1075, Schiff Reply, ¶¶55-56.

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Ex. 1013, p. 3238; Ex. 1004, Schiff ¶¶ 57-62; Ex. 1075, Schiff Reply, ¶¶55-56.

Prior Art: Worzalla (Ex. 1013)

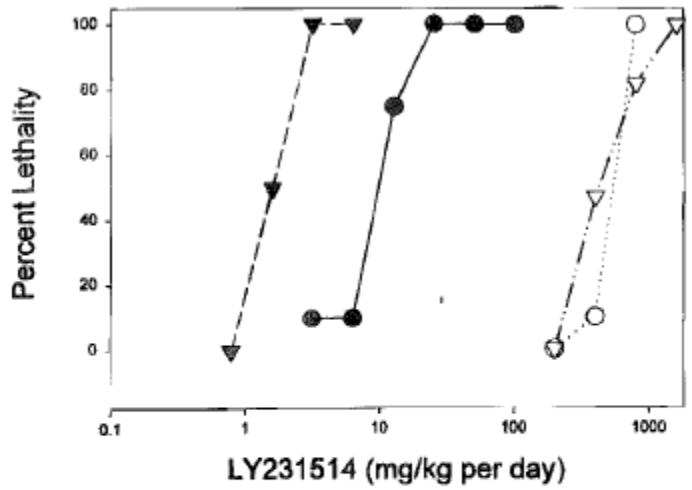


Figure 1. *The toxicity of LY231514 in mice is increased by a folate-deficient diet. DBA/2 and CDl nu/nu mice were fed either a standard laboratory diet (○ and ▽, respectively) or a folate-deficient diet for 2 weeks prior to the first dose of LY231514 (● and ▼, respectively) and for the duration of the study. Groups of mice (> 10 animals/group) on each diet were given 10 daily doses of LY231514 i.p. at the indicated doses. The data present the percent lethality within 3 weeks after the last dose of LY231514.*

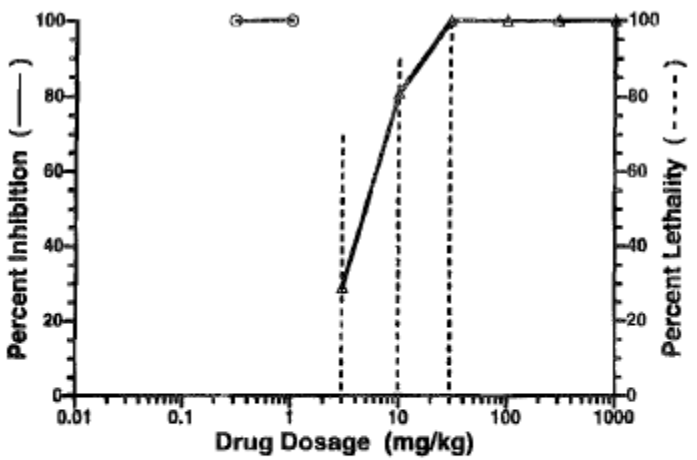
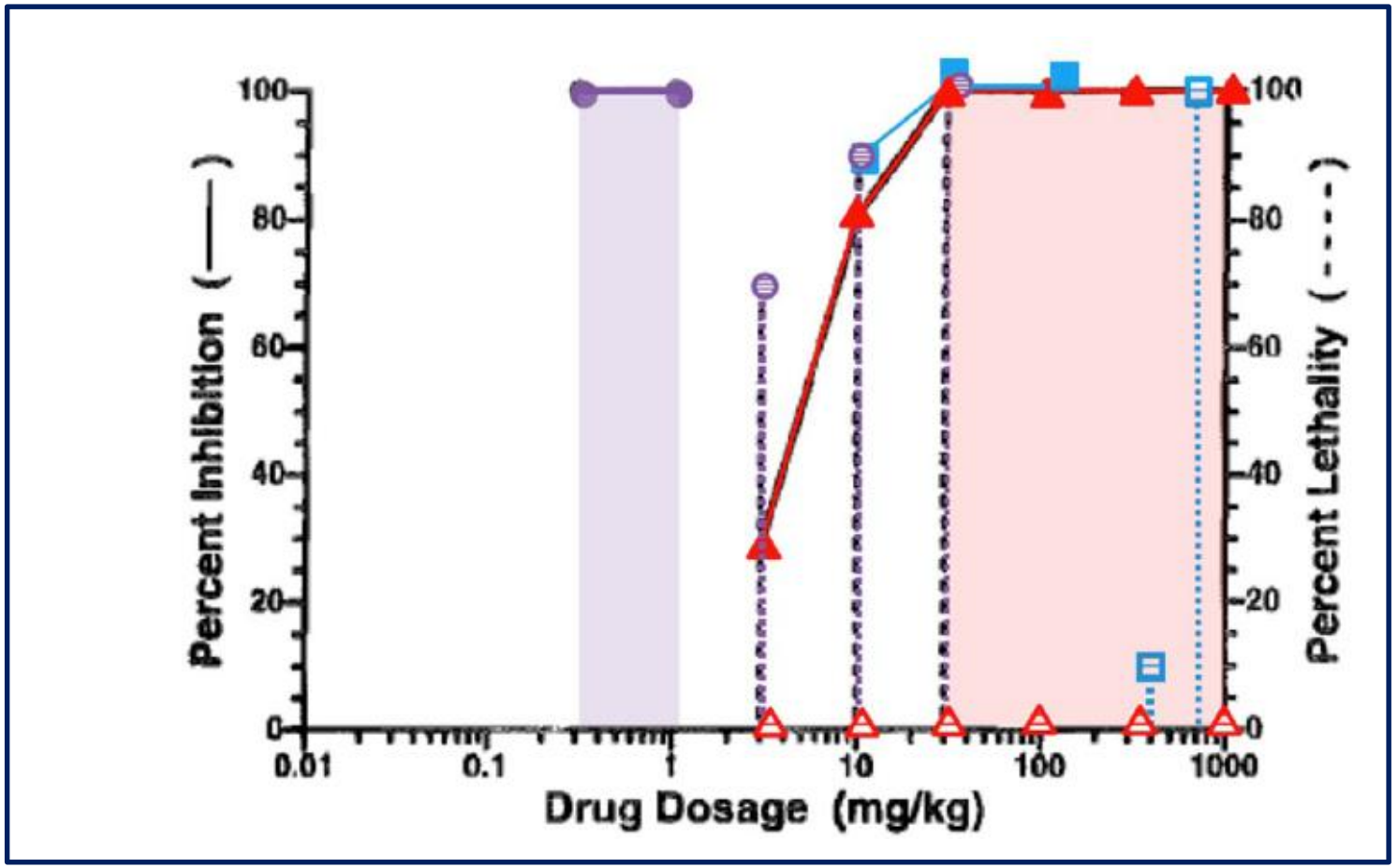


Figure 2. *Antitumor activity of LY231514 therapy (i.p., qd ×10) against L5178Y/TK- /HX- lymphoma for mice on low folate diet with no folate supplementation (○) and for mice on low folate diet that received 15 mg/kg/day daily folate supplementation (△). Vertical dashed lines represent percent lethality in mice on low folate diet with no folate supplementation. No lethality was observed in mice that received folate supplementation.*

Ex. 1013, p. 3238; Ex. 1004, Schiff ¶¶ 57-62; Ex. 1075, Schiff Reply, ¶¶55-56.

Prior Art: Worzalla (Ex. 1013)



mice on low folate diet (LFD) with no folate supplementation

mice on LFD with 15mg/kg/day folate supplementation

standard diet mice

vertical dashed lines = % lethality, dashed shapes indicate maximum % lethality

Shading = range (if any) with 100% tumor inhibition, 0% lethality

Ex. 1013, p. 3238; Ex. 1004, Schiff ¶¶57-62; Ex. 1075, Schiff Reply ¶¶57-58; Ex. 1074, Chabner Dep. 121:5-23, 129:13-19 (citing Ex. 1067, Demonstrative).

Prior Art: Worzalla (Ex. 1013)

ANTICANCER RESEARCH *18*: 3235-3240 (1998)

Role of Folic Acid in Modulating the Toxicity and Efficacy of the Multitargeted Antifolate, LY231514

JOHN F. WORZALLA, CHUAN SHIH and RICHARD M. SCHULTZ

Cancer Research Division, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285, U.S.A.

Abstract. *We studied the effects of folic acid on modulating the toxicity and antitumor efficacy of LY231514. Using several human tumor cell lines adapted to growth in low folate medium, folic acid was shown to be 100- to 1000-fold less active than folinic acid at protecting cells from LY231514-induced cytotoxicity. The lethality of LY231514 was compared in mice maintained on standard diet or low folate diet. The LD50 occurred at 60- and 250-fold lower doses of LY231514 in DBA/2 and CD1 nu/nu mice, respectively, maintained on low folate diet compared to standard diet. The L5178Y/TK-/HX- murine lymphoma was much more sensitive to the antitumor action of LY231514 compared to wild type L5178Y-S tumors. For mice on low folate diet, LY231514 at 0.3 and 1 mg/kg (qd x 10, i.p.) produced 100% inhibition of L5178Y/TK-/HX- lymphoma growth, and significant lethality occurred at ≥ 3 mg/kg. For mice on standard diet, LY231514 produced >95% inhibition of tumor growth at 30 to 300 mg/kg, but all mice died at 800 mg/kg. Folic acid supplementation was demonstrated to preserve the antitumor activity of LY231514 while reducing toxicity. The combination of folic acid with LY231514 may provide a mechanism for enhanced clinical antitumor selectivity.*

LY231514 is a structurally novel antifolate antimetabolite that possesses the unique 6-5-fused pyrrolo[2,3-d]pyrimidine nucleus (1) instead of the more common 6-6-fused pteridine or quinazoline ring structure. The primary mode of antitumor activity for LY231514 has previously been ascribed to inhibition of thymidylate synthase (TS) (1, 2). However, several lines of evidence suggest that multiple enzyme-inhibitory mechanisms are involved in cytotoxicity, hence the acronym MTA (multitargeted antifolate): 1) the reversal pattern for MTA in human leukemia and colon carcinoma cell lines demonstrates that although TS may be a major site of action for LY231514 at concentrations near the IC50, higher concentrations can lead to inhibition of dihydrofolate reductase (DHFR) and/or other enzymes along the purine de novo pathway (3); 2) MTA is an excellent substrate for folylpolyglutamate synthetase, and the K_i values of the pentaglutamate of LY231514 are 1.3, 7.2, and 65 nM for inhibition against TS, DHFR and glycinamide ribonucleotide formyltransferase (GARFT), respectively (3); 3) intracellular concentrations of LY231514 and its polyglutamates can exceed 40 μM in CCRF-CEM cells when ^{3}H-labeled LY231514 was used (R.M. Schultz, unpublished observation); and 4) early clinical studies demonstrated that patients who had previously failed to respond to ZD1694 and 5-fluorouracil/leucovorin treatment responded to LY231514 (4; DA Rinaldi, personal communication).

Several animal studies have indicated that folic acid supplementation in combination with antifolate cancer therapy can prevent delayed toxicity and enhance the therapeutic potential of the GARFT inhibitor lometrexol (5, 6) and the TS inhibitor 1843U89 (7). Unexpected delayed cumulative toxicity was observed in phase I studies with lometrexol, including thrombocytopenia, anemia, and mucositis (8). Additional clinical studies demonstrated the protective effects of folic acid against lometrexol toxicity in humans (9). Morgan and coworkers (10) concluded that a daily supplement of 1 mg of folic acid during low-dose methotrexate therapy in patients with rheumatoid arthritis was useful in lessening toxicity without altering efficacy. In the present communication, we investigated the effects of folic acid on the antitumor activity and lethality of LY231514 in mice.

Materials and Methods

Reagents. Folic acid, folinic acid (leucovorin), and 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The disodium salt of LY231514 was synthesized at Eli Lilly and Co. (1).

Cell lines. Human CCRF-CEM leukemia cells were obtained from St. Jude Children's Research Hospital (Memphis, TN, USA). Human IGROV1 ovarian carcinoma cells were generously supplied by Dr.

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Key Words: LY231514, antitumor activity, antifolate, folic acid.

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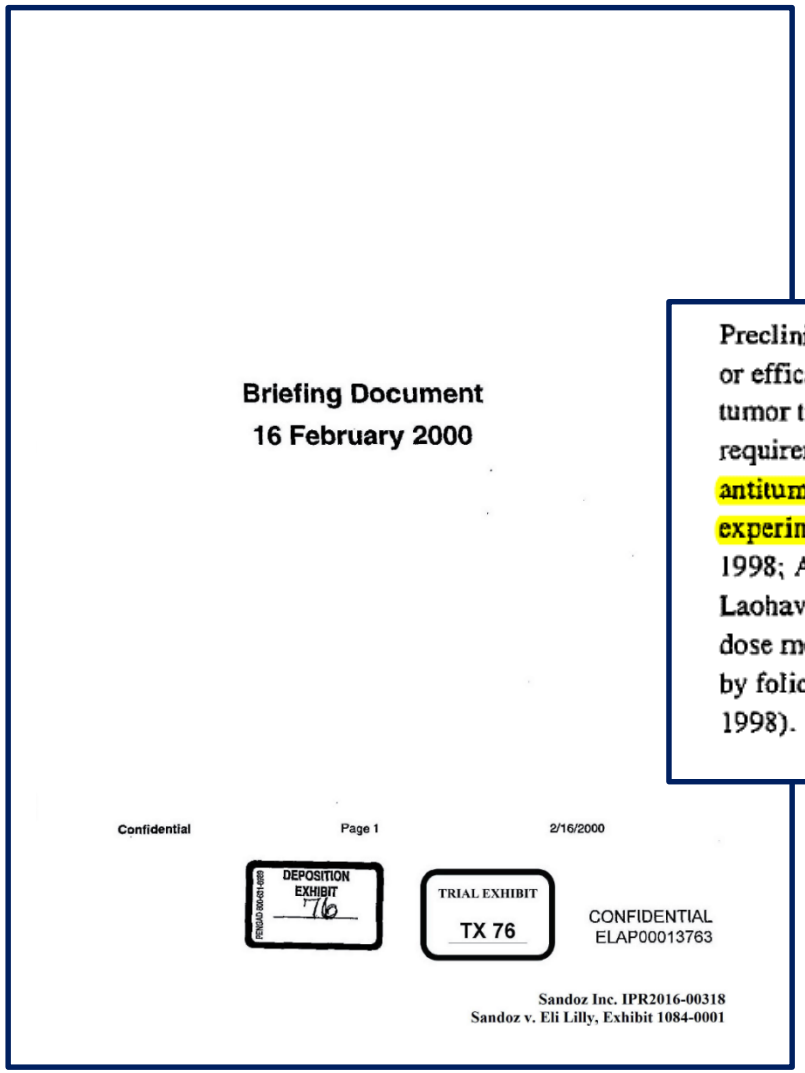
Exhibit 1013-0001

Abstract. *We studied the effects of folic acid on modulating the toxicity and antitumor efficacy of LY231514. Using several human tumor cell lines adapted to growth in low folate medium, folic acid was shown to be 100- to 1000-fold less active than folinic acid at protecting cells from LY231514-induced cytotoxicity. The lethality of LY231514 was compared in mice maintained on standard diet or low folate diet. The LD50 occurred at 60- and 250-fold lower doses of LY231514 in DBA/2 and CD1 nu/nu mice, respectively, maintained on low folate diet compared to standard diet. The L5178Y/TK-/HX- murine lymphoma was much more sensitive to the antitumor action of LY231514 compared to wild type L5178Y-S tumors. For mice on low folate diet, LY231514 at 0.3 and 1 mg/kg (qd x 10, i.p.) produced 100% inhibition of L5178Y/TK-/HX- lymphoma growth, and significant lethality occurred at ≥ 3 mg/kg. For mice on standard diet, LY231514 produced >95% inhibition of tumor growth at 30 to 300 mg/kg, but all mice died at 800 mg/kg. Folic acid supplementation was demonstrated to preserve the antitumor activity of LY231514 while reducing toxicity. The combination of folic acid with LY231514 may provide a mechanism for enhanced clinical antitumor selectivity.*

Ex. 1013, p. 3235; Ex. 1004, Schiff ¶¶ 57-62;
Ex. 1075, Schiff Reply, ¶ 56-58.

Lilly's Statements to FDA about Worzalla



Briefing Document
16 February 2000

Confidential Page 1 2/16/2000

DEPOSITION EXHIBIT 76

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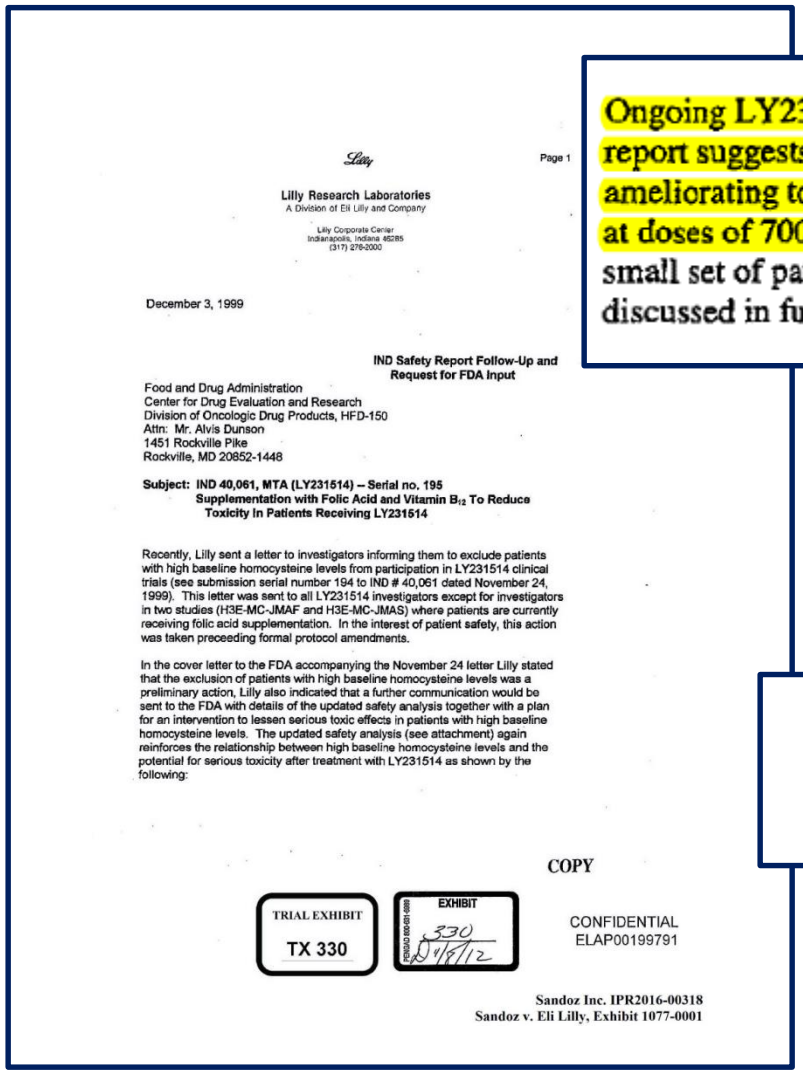
Sandoz Inc. IPR2016-00318
Sandoz v. Eli Lilly, Exhibit 1084-0001

Preclinical and clinical studies evaluating the impact of dietary folic acid on the toxicity or efficacy of antifolates such as LY231514 and lometrexol have been reported. Because tumor tissue and normal tissue, such as bone marrow, presumably have different folate requirements, it is possible to decrease the toxicity to healthy tissue while maintaining antitumor effect through careful adjustment of folic acid intake. This has been shown in experimental systems for LY231514 and another antifolate, lometrexol (Worzalla et al. 1998; Alati et al. 1996) and in clinical trials with lometrexol (Young et al. 1992; Laohavinij et al. 1996). In addition, it has been clinically observed that the efficacy of low dose methotrexate used in the treatment of rheumatoid arthritis is not negatively affected by folic acid supplementation, while an improvement in toxicity is seen (Morgan et al. 1998).

Ex. 1084, Feb. 16, 2000 Briefing Document at ELAP0013767; Paper 49, Sandoz Reply at 8-9.

Lilly's Statements to FDA about Hammond



Lilly

Page 1

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A Division of Eli Lilly and Company

Lilly Corporate Center
Indianapolis, Indiana 46285
(317) 276-2000

December 3, 1999

IND Safety Report Follow-Up and Request for FDA Input

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncologic Drug Products, HFD-150
Attn: Mr. Alvis Dunson
1451 Rockville Pike
Rockville, MD 20852-1448

Subject: IND 40,061, MTA (LY231514) – Serial no. 195
Supplementation with Folic Acid and Vitamin B_{12} To Reduce Toxicity In Patients Receiving LY231514

Recently, Lilly sent a letter to investigators informing them to exclude patients with high baseline homocysteine levels from participation in LY231514 clinical trials (see submission serial number 194 to IND # 40,061 dated November 24, 1999). This letter was sent to all LY231514 investigators except for investigators in two studies (H3E-MC-JMAF and H3E-MC-JMAS) where patients are currently receiving folic acid supplementation. In the interest of patient safety, this action was taken preceeding formal protocol amendments.

In the cover letter to the FDA accompanying the November 24 letter Lilly stated that the exclusion of patients with high baseline homocysteine levels was a preliminary action, Lilly also indicated that a further communication would be sent to the FDA with details of the updated safety analysis together with a plan for an intervention to lessen serious toxic effects in patients with high baseline homocysteine levels. The updated safety analysis (see attachment) again reinforces the relationship between high baseline homocysteine levels and the potential for serious toxicity after treatment with LY231514 as shown by the following:

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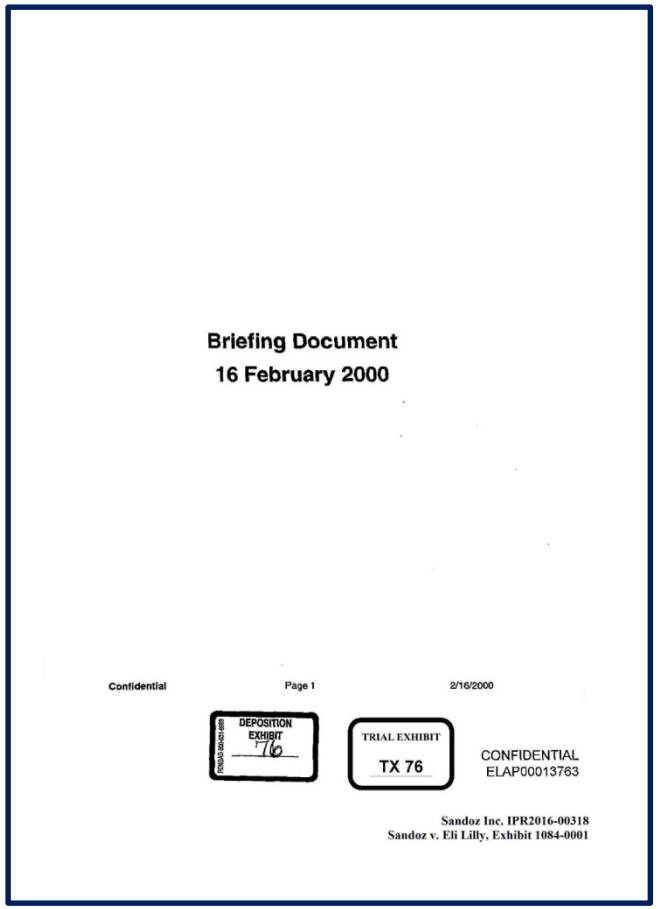
Ongoing LY231514 trials include a phase I study of LY231514 and folic acid. An interim report suggests that folic acid supplementation in this study permits dose escalation by ameliorating toxicity since heavily and minimally pretreated patients tolerate LY231514 at doses of 700 and 925 mg/m^2 respectively [10]. In a phase II trial in gastric cancer, a small set of patients has also received folic acid supplementation. This trial will be discussed in further detail in Section 3.

Ex. 1077, Dec. 3, 1999 Letter Lilly to FDA at ELAP00199798, -814; Paper 49, Sandoz Reply at 8-9.

10. Hammond L, Villalona – Calero M, Eckhardt S G, Siu L, Hidalgo M, Thornton D, Walling J, Baker S, Coltman C, Von Hoff D, Rowinsnky E. A phase I and pharmacokinetic (PK) study of the mutitargeted antifolate (MTA, LY231514) with folic acid. Ann Oncol 9 suppl 4 abstract 620

Lilly's Statements to FDA about Worzalla



Briefing Document
16 February 2000

Confidential Page 1 2/16/2000

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Sandoz v. Eli Lilly, Exhibit 1084-0001

Cancer – Preclinical Data

Worzalla and coworkers have studied the effects of folic acid on the toxicity and antitumor activity of LY231514 in the in vitro and in vivo settings. In a number of human tumor cell lines, folic acid protected cells from cytotoxicity at concentrations 100- to 1000-fold higher than those required for folinic acid protection, indicating that the action of LY231514 is less sensitive in vitro to folic acid than it is to folinic acid. They also found that in mice fed a low folate diet (LFD), tumor growth inhibition was complete at LY231514 doses of 0.9 to 3.0 mg/m^2, with 100% lethality occurring at LY231514 doses of 9.0 mg/m^2 or higher. Mice receiving the same LFD who were supplemented with high doses of folic acid at 15 mg/kg/day (a dose approximately 10-fold greater than that in the normal diet) experienced complete tumor growth inhibition at LY231514 doses of 90 to 3000 mg/m^2 without any lethality. Mice on the standard diet (approximately one tenth of the folic acid given to the supplemented mice) saw a virtually identical dose response, but greater lethality, with 100% lethality occurring at 2400 mg/m^2 (Worzalla et al. 1998).

Doses of LY231514 for Maximum Antitumor Activity and Lethality in Mice

Diet	Doses[a] of LY231514 Where Maximum Antitumor Activity is Observed	Doses[a] of LY231514 Where Lethality is Observed
Standard Diet (Daily folic acid intake = 4.5 mg/m^2)	From 90 to 1200	2400 (100% lethality)
LFD + 15 mg/kg Folic Acid (Daily folic acid intake = 45 mg/m^2)	From 90 to 3000	(No lethality seen up to 3000)

[a]Doses in mg/m^2/day

These data show that antitumor activity is virtually identical in mice receiving a standard diet to that in mice receiving a 10-fold increase in daily folic acid. Mice receiving the extra folic acid also showed a decreased lethality at higher doses of LY231514. These data support the hypothesis that folic acid supplementation can protect healthy tissue from the toxic effects of LY231514 with retention of antitumor activity.

Ex. 1084, Feb. 16, 2000 Briefing Document at ELAP0013768-69; Paper 49, Sandoz Reply at 8-9.

Prior Art Disclosures

FOLIC ACID + VITAMIN B_{12}

Prior Art: Calvert (Ex. 1007) and Pemetrexed Toxicity, Homocysteine, and Vitamins

An Overview of Folate Metabolism: Features Relevant to the Action and Toxicities of Antifolate Anticancer Agents

Hilary Calvert

SINCE the observation of reduced folate levels in children with leukemia made by Farber et al[1] in the 1940s, the study of folic acid metabolism and the action of antifolate drugs has been intimately linked to the development of cancer therapeutics. Folic acid plays a role in a wide range of metabolic pathways in various species. In humans it is an essential vitamin and functions primarily in the processes involved in cellular proliferation and amino acid metabolism. This review will focus mainly on those aspects of mammalian folate metabolism relevant to cell proliferation since these are the most germane to the use of antifolates in cancer therapy. The textbook by R.L. Blakley[2] is a comprehensive work covering all aspects of folate metabolism.

ASPECTS OF FOLATE METABOLISM

Folate Pathways Associated With Cell Proliferation

Folic acid functions mainly in its fully reduced form, 5,6,7,8-tetrahydrofolate (FH_4; Fig 1). FH4 serves as a carrier for one-carbon moieties within the cell. These are obtained from a variety of sources that include serine. In this reaction, serine hydroxymethyl transferase forms 5,10-methylene tetrahydrofolate (CH_2FH_4) while converting serine to glycine (Fig 2). CH_2FH_4 may be converted within the cell to one-carbon carrying folate derivatives of various oxidation states. One of these, 10-formyl tetrahydrofolate, is the substrate for two enzymes involved in the de novo synthesis of purines. These are glycinamide ribonucleotide formyl transferase (GARFT) and aminoimidazole carboxamide ribonucleotide formyl transferase (AICARFT). Thus, two of the carbon atoms in the purine skeleton are derived from folate. The folate-dependent reactions of purine synthesis use the carbon atom from the 10-formyl group and release unsubstituted tetrahydrofolate as the folate product. Thus, the folate molecule can then acquire another carbon atom from serine and continue to cycle through GARFT and AICARFT, allowing continued purine synthesis without any overall consumption of folate. CH_2FH_4 is also the substrate for the enzyme thymidylate synthase (TS). Thymidylate synthase converts deoxyuridine monophosphate into thymidine monophosphate and is a key enzyme involved in cell proliferation because it is the rate-limiting step in the de novo synthesis of thymidylate, which is required exclusively for DNA synthesis. The folate product of TS is not tetrahydrofolate, but the oxidized form, dihydrofolate (FH_2). This product cannot continue to function in folate metabolism but is converted back to FH_4 by the enzyme dihydrofolate reductase (DHFR).

The Role of Folate and Antifolate Polyglutamates

Folic acid possesses a glutamate residue shown at the right-hand side of the folate structures in Fig 1. Naturally occurring folates within the cell are converted to polyglutamate forms by the addition of glutamate residues via a γ-peptide linkage. Antifolates that possess a glutamate residue (known as classical antifolates) are also frequently converted into their corresponding polyglutamate forms. The process of polyglutamation is accomplished by the enzyme folylpoly-γ-glutamate synthetase. This reaction is illustrated in Fig 3 using the antifolate LY231514 (MTA) as an example. The process is analogous for natural folates and many other classical antifolates. In Fig 3, the carboxylate groups of the glutamic acid residue are shown in their ionized form, carrying a negative charge, showing that polyglutamation increases the overall negative charge on the folate molecule by one unit for each additional glutamate. The negatively charged polyglutamates cannot cross the cell membrane and are therefore retained and concentrated within the cell. This is probably the major physiologic role of polyglutamation. Cells that are deficient in folylpoly-γ-glutamate synthetase are auxotrophic for the end products of

From the Cancer Research Unit, Department of Oncology, University of Newcastle upon Tyne.
Sponsored by Eli Lilly and Company.
Dr Calvert is a consultant for and has received research funding from Eli Lilly and Company and Zeneca.
Address reprint requests to Hilary Calvert, MD, Cancer Research Unit, Department of Oncology, Framlington Place, University of Newcastle upon Tyne, NE2 4HH.
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0093-7754/99/2602-0602$10.00/0

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Exhibit 1007-0001

Clinical Measurement of Functional Folate Status

Although the effect of folic acid supplementation on reducing the toxicity of antifolate drugs (particularly the GARFT inhibitors) is clear, it always has been difficult to correlate antifolate-induced toxicity with pretreatment folate levels. One possible explanation for this is that the folate levels do not adequately reflect the functioning of folic acid within proliferating cells at the time of measurement. In addition to the pathways discussed so far, folic acid is also involved in cellular methylation reactions by virtue of its role in methionine synthesis. CH_2FH_4 can be reduced to 5-methyltetrahydrofolate (Fig 1). This is a substrate for the enzyme methionine synthase, which uses the methyl group to convert homocysteine to methionine. Methionine in turn takes part in cellular methylation reactions regenerating homocysteine. Methionine synthase is B_{12}-dependent but also uses 5-methyltetrahydrofolate as the co-substrate. Thus, any functional deficiency either in B_{12} or folate will result in reduction in the flux through methionine synthase and a consequent increase in the plasma level of homocysteine[16] (Fig 8). The measurement of pretreatment plasma homocysteine has proved to be a sensitive way of predicting the toxicity of MTA.[17]

Ex. 1007, Calvert at 8-9; Ex. 1004, Schiff ¶¶71-73.

Prior Art: Calvert (Ex. 1007) and Pemetrexed Toxicity, Homocysteine, and Vitamins

Clinical Measurement of Functional Folate Status

Although the effect
tion on reducing the
(particularly the GAI
always has been diffic
induced toxicity with
One possible explanati
levels do not adequate
folic acid within proli
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strate for the enzyme
uses the methyl group
methionine. Methionine in turn tak
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teine. Methionine synthase
also uses 5-methyltetra
strate. Thus, any f
B_{12} or folate
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increase in the plasma level of homocysteine[16]
(Fig 8). The measurement of pretreatment plasma
homocysteine has proved to be a sensitive
predicting the toxicity of MTA.[17]

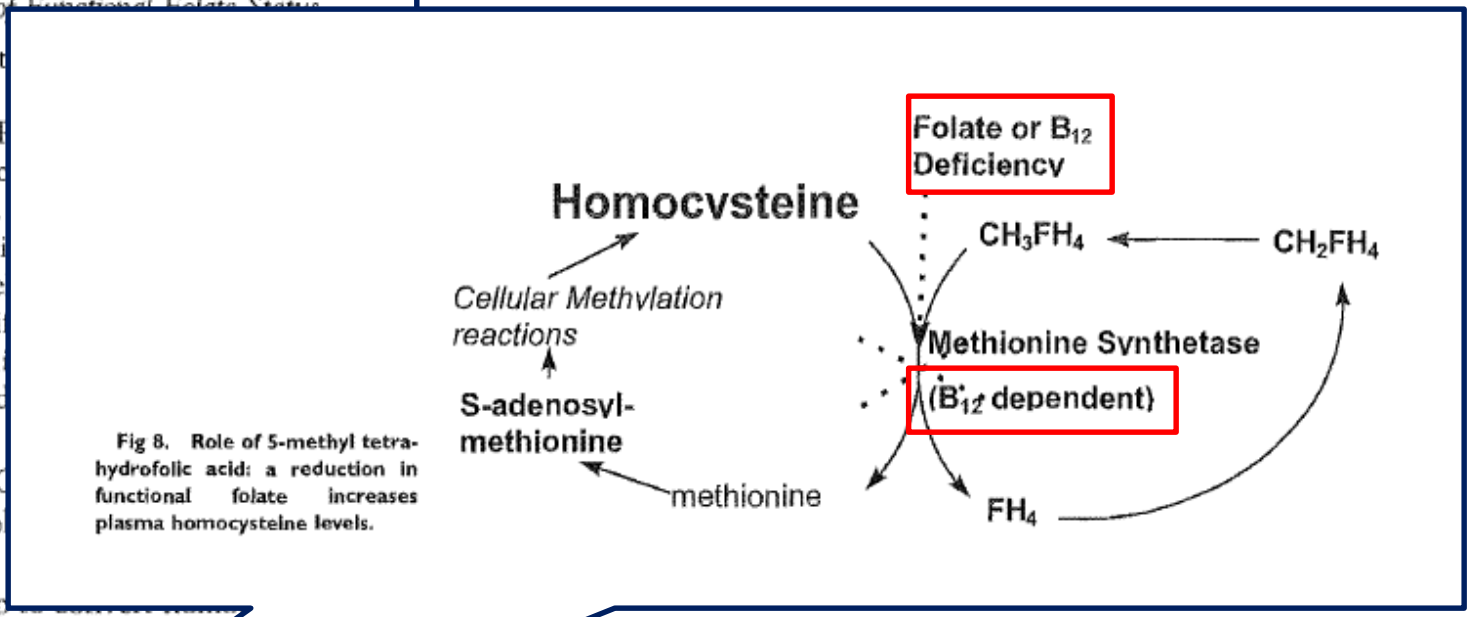

Fig 8. Role of 5-methyl tetrahydrofolic acid: a reduction in functional folate increases plasma homocysteine levels.

17. Niyikiza C, Walling J, Thornton D, et al: LY231514 (MTA): Relationship of vitamin metabolite profile to toxicity. Proc Am Assoc Clin Oncol 34:2139, 1998 (abstr)

Ex. 1007, Calvert at 8-9; Schiff ¶¶71-73.

Prior Art: Calvert (Ex. 1007)

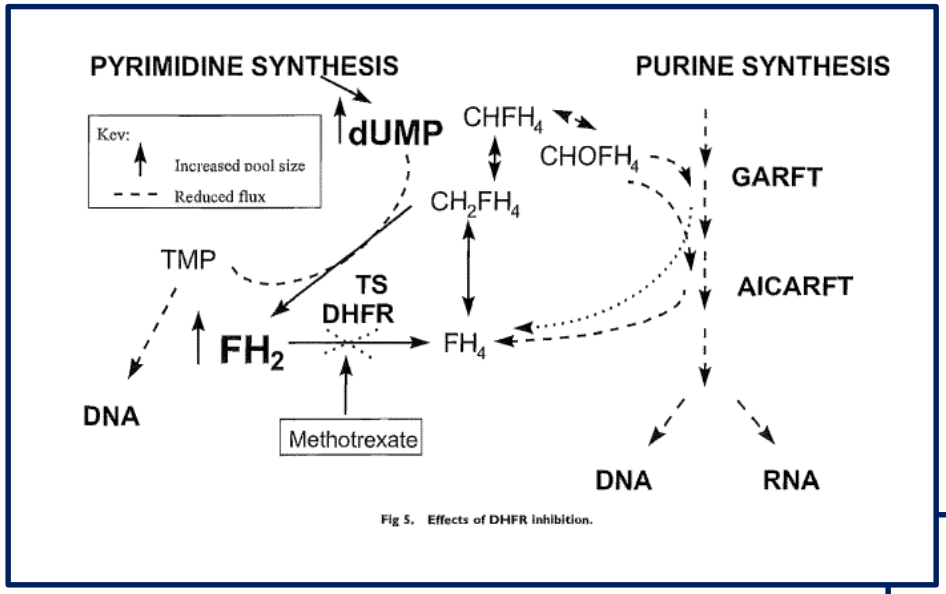

Fig 5. Effects of DHFR inhibition.

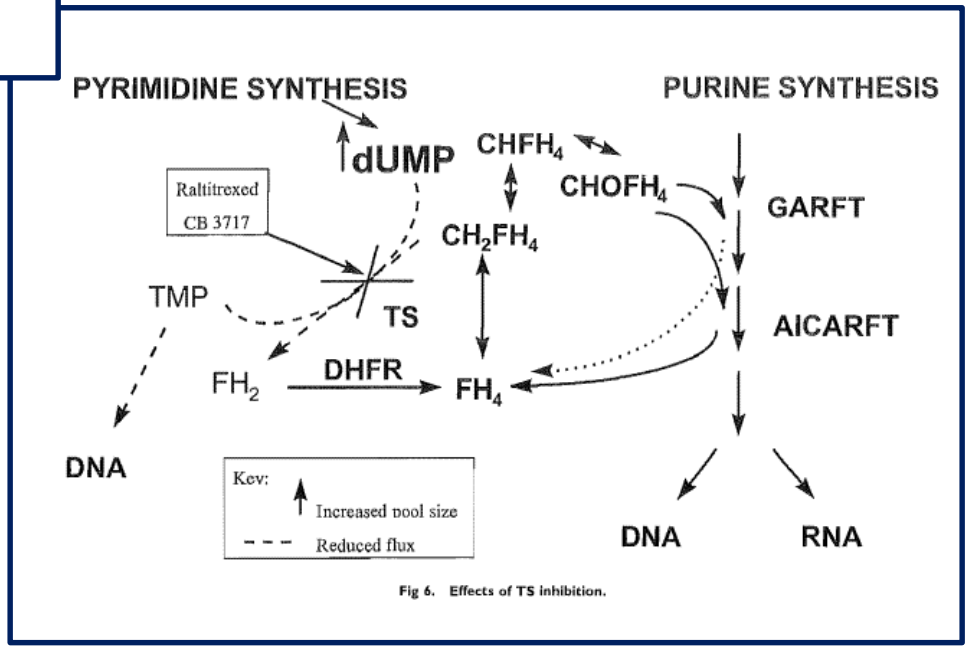

Fig 6. Effects of TS inhibition.

Ex. 1007, Calvert at 7-8; Ex. 1004, Schiff ¶¶ 71-73; Ex. 1075, Schiff Reply, ¶¶75-76.

Prior Art: Niyikiza Abstracts

Niyikiza I (Ex. 1006)

609P **MTA (LY231514): Relationship of vitamin metabolite profile, drug exposure, and other patient characteristics to toxicity**

C. Niyikiza, S. Baker, R. Johnson, J. Walling, D. Seitz, R. Allen. *Lilly Research Laboratories, Indiana, USA; Cancer Treatment and Research Center, Texas, USA; Univ of Colorado Health Sciences Center, Colorado, USA*

Introduction: MTA is a novel multitargeted antifolate with inhibitory activity against multiple enzymes. Phase I/II studies have shown activity in a variety of tumors. Historical data on other antifolates have suggested that a patient's nutritional status may play a role in the likelihood of experiencing severe toxicity. The purpose of this study was to assess the relationship of vitamin metabolites, drug exposure, and other prespecified baseline patient characteristics to toxicity following treatment with MTA.

Methods: Homocysteine (Hcys), cystathionine and methylmalonic acid were measured in 139 phase II patients with tumors of the colon, breast, pancreas, and esophagus at baseline and once each cycle thereafter. Stepwise regression modeling, multivariate analysis of variance, and discriminant analysis were implemented to determine which predictors might correlate with severe toxicity after one course of MTA. Prognostic factors considered were age, gender, prior treatment, baseline albumin, liver enzymes, ANC, platelets, vitamin metabolites, and AUC.

Results: Statistically significant predictors of Grade 4 neutropenia (n=21 pts) were albumin ($p = 0.0006$) and Hcys ($p = 0.0012$), while Grade 4 thrombocytopenia (n=8) was highly predicted by Hcys ($p < 0.0001$) and pre-treatment AST ($p = 0.0012$). Hcys $\geq 10\mu M$ predicted Grade 4 neutropenia in cycle one 75% of the time. Grade 4 neutropenia was predicted by Hcys alone in 70% of cases. Hcys and albumin levels did not appear to change from baseline during treatment with MTA. While AUC was not found to be a predictor of toxicity, little variability was observed in AUC. Maximum values were still below AUC values related to hematologic toxicity in phase I studies.

Conclusions: Toxicities resulting from treatment with MTA appear to be predictable from pretreatment homocysteine levels. Elevated baseline homocysteine levels ($\geq 10\mu M$) highly correlate with severe hematologic and nonhematologic toxicities following treatment with MTA. Homocysteine was found to be better than albumin at predicting toxicity. These results apply to the tumor types studied. Further studies are underway in patients with renal impairment or patients who received prior cisplatin.

Ex. 1006, Niyikiza I (ESMO, Nov. 6-10, 1998); Ex. 1004, Schiff ¶¶67-68; Ex. 1075, Schiff Reply, ¶¶66-69.

Niyikiza II (Ex. 1016)

*2139

LY231514 (MTA): RELATIONSHIP OF VITAMIN METABOLITE PROFILE TO TOXICITY. *C. Niyikiza, J. Walling, D. Thornton, D. Seitz, and R. Allen. Eli Lilly and Company, Indianapolis, IN, and Univ of Colorado Health Science Center, Denver, CO.*

LY231514 (MTA) is a new generation multitargeted antifolate antimetabolite with inhibitory activity against thymidylate synthase, dihydrofolate reductase and glycinamide ribonucleotide formyl transferase. Of a total of 246 patients (pts) in phase II trials treated with MTA (600 mg/m^2 IV over 10 minutes once every 21 days) 118 pts also had vitamin metabolites measured. Because earlier studies with other antifolates had suggested that nutritional status may play a role in the likelihood that a patient will experience severe toxicity, levels of the vitamin metabolites homocysteine, cystathionine and methylmalonic acid were measured at baseline and once each cycle thereafter. A multivariate statistical analysis of the data was conducted in order to determine which among a set of pre-specified predictors (creatinine clearance, albumin levels, liver enzyme levels, and vitamin metabolites) might correlate with toxicity. There was a strong correlation between baseline homocysteine levels and the development of the following toxicities at any time during the study: CTC Grade 4 neutropenia (57 pts, $p < 0.0001$), Grade 4 thrombocytopenia (13 pts, $p < 0.0001$), Grade 3 or 4 mucositis (8 pts, $p < 0.0003$), and Grade 3 or 4 diarrhea (8 pts, $p < 0.004$). Cystathionine levels did not correlate with hematologic toxicity or mucositis but were moderately correlated with fatigue ($p < 0.04$). Maximum cystathionine levels doubled from baseline during treatment with MTA. No correlation between toxicity (CTC Grades as defined above) and the remaining pre-specified predictors was seen. Toxicity was seen in all patients with homocysteine levels above a threshold concentration of 10 μM. A correlation over time between homocysteine levels and CTC Grade 4 neutropenia and thrombocytopenia and CTC Grade 3 or 4 mucositis was also observed, but only in the first two cycles of treatment. Maximum homocysteine levels did not appear to change from baseline during treatment with MTA.

Ex. 1016, Niyikiza II (ASCO, May 16-19, 1998); Ex. 1004, Schiff ¶69; Ex. 1075, Schiff Reply, ¶¶66-69.

Prior Art: Niyikiza Abstracts - Lilly's Claim that Niyikiza II (ASCO, Ex. 1016) Suggests A Lack Of B_{12} Deficiency

4 diarrhea (8 pts, $p < 0.004$). Cystathionine levels did not correlate with hematologic toxicity or mucositis but were moderately correlated with fatigue ($p < 0.04$). Maximum cystathionine levels doubled from baseline

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*2139

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LY231514 (MTA) is a new generation multitargeted antifolate antimetabolite with inhibitory activity against thymidylate synthase, dihydrofolate reductase and glycinamide ribonucleotide formyl transferase. Of a total of 246 patients (pts) in phase II trials treated with MTA (600 mg/m^2 IV over 10 minutes once every 21 days) 118 pts also had vitamin metabolites measured. Because earlier studies with other antifolates had suggested that nutritional status may play a role in the likelihood that a patient will experience severe toxicity, levels of the vitamin metabolites homocysteine, cystathionine and methylmalonic acid were measured at baseline and once each cycle thereafter. A multivariate statistical analysis of the data was conducted in order to determine which among a set of pre-specified predictors (creatinine clearance, albumin levels, liver enzyme levels, and vitamin metabolites) might correlate with toxicity. There was a strong correlation between baseline homocysteine levels and the development of the following toxicities at any time during the study: CTC Grade 4 neutropenia (57 pts, $p < 0.0001$), Grade 4 thrombocytopenia (13 pts, $p < 0.0001$), Grade 3 or 4 mucositis (8 pts, $p < 0.0003$), and Grade 3 or 4 diarrhea (8 pts, $p < 0.004$). Cystathionine levels did not correlate with hematologic toxicity or mucositis but were moderately correlated with fatigue ($p < 0.04$). Maximum cystathionine levels doubled from baseline during treatment with MTA. No correlation between toxicity (CTC Grades as defined above) and the remaining pre-specified predictors was seen. Toxicity was seen in all patients with homocysteine levels above a threshold concentration of 10 μM. A correlation over time between homocysteine levels and CTC Grade 4 neutropenia and thrombocytopenia and CTC Grade 3 or 4 mucositis was also observed, but only in the first two cycles of treatment. Maximum homocysteine levels did not appear to change from baseline during treatment with MTA.

Ex. 1016, Niyikiza II (ASCO, May 16-19, 1998); Ex. 1004, Schiff ¶69; Ex. 1075, Schiff Reply, ¶¶66-69.

Prior Art: Niyikiza Abstracts - Drs. Chabner's and Zeisel's Testimony

Dr. Chabner

So it's your opinion, one of ordinary skill in the art, in June of 1999 that when Dr. Niyikiza reports that no correlation with MMA was seen, that what he really meant was none exists? Is that your opinion?

A. No. You couldn't say that about anything. Nothing was seen in this study. And the same thing is true of cystathionine. Nothing -- it wasn't seen in this study. The point I'm trying to make is that neither one is an absolute statement about whether it exists in nature if you did a large enough study. You can't ever -- scientifically you can't exclude something that way. All you can say is I didn't see it in the study.

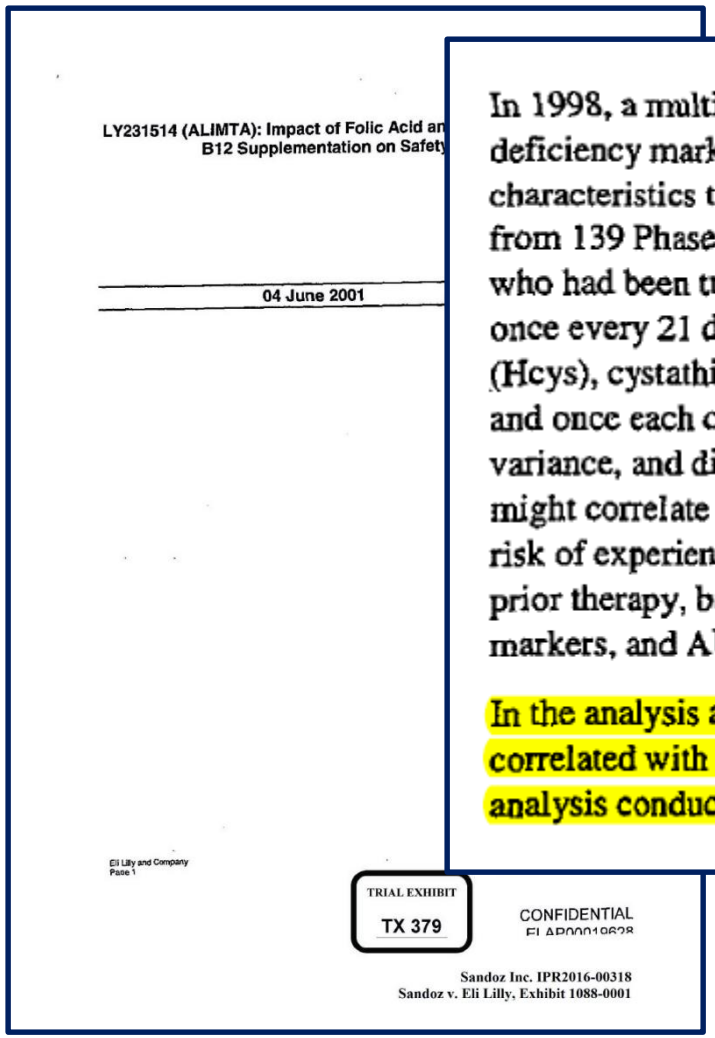
Ex. 1074, Chabner Dep. Tr. 152:23-153:13; Paper 49, Sandoz Reply at 12.

Dr. Zeisel

However, in the presence of normal MMA, I believe that Niyakiza does not support the conclusion that B12 is -- B12 deficiency is the cause. It just says that, at least in this group of patients, I can't say it is and I can't rule out it isn't.

Ex. 1086, Zeisel Dep. Tr. 116:19-20; Paper 49, Sandoz Reply at 12.

Lilly's Statements to FDA about the Niyikiza Abstracts



LY231514 (ALIMTA): Impact of Folic Acid an
B12 Supplementation on Safety

04 June 2001

Eli Lilly and Company
Page 1

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Sandoz v. Eli Lilly, Exhibit 1088-0001

In 1998, a multivariate analysis was conducted to assess the relationship of vitamin deficiency markers, LY231514 exposure, and pre-specified baseline patient characteristics to toxicity following therapy with LY231514 [4]. Data were examined from 139 Phase 2 patients with tumors of the colon, breast, pancreas, and esophagus who had been treated with single agent LY231514 at 600 mg/m2 IV over 10 minutes once every 21 days. These patients had vitamin-deficiency markers of homocysteine (Hcys), cystathionine, and methylmalonic acid levels measured in plasma at baseline and once each cycle thereafter. Stepwise regression modeling, multivariate analysis of variance, and discriminant analysis were implemented to determine which predictors might correlate with severe toxicity, and to predict which patients might be at high risk of experiencing such toxicity. Prognostic factors considered were age, gender, prior therapy, baseline albumin, liver enzymes, ANC, platelets, vitamin deficiency markers, and AUC.

In the analysis above, the B12 deficiency marker, methylmalonic acid, was highly correlated with homocysteine and was therefore removed from the initial multivariate analysis conducted in 1998 to eliminate issues of colinearity.

Ex. 1088, June 4, 2001 Lilly Report, ELAP00019630; Paper 49, Sandoz Reply at 13.

Vitamin B_{12} and Methylmalonic Acid (MMA)

A Perhaps 5 percent of people with low B12 do not have abnormal MMA.

Ex. 1086, Zeisel Dep. 95:11-12.; Ex. 1075, Schiff Reply ¶68; Ex. 1091, Stover Reply ¶42

Q. So I just want to make sure I understand something. The -- one of ordinary skill in the art in June of 1999, their takeaway from the Allen paper is that approximately 5 percent of those individuals who are Vitamin B12 deficient --

A. Are low levels.

Q. -- low levels of B12 do not have elevated MMA levels?

A. Right.

Ex. 1074, Chabner Dep. 206:12-21; Ex. 1075, Schiff Reply ¶68

Patients in whom serum levels of both folate and Cbl are low are frequently encountered clinically and often pose a diagnostic problem. Although in more than 75% of patients with Cbl deficiency in our experience, serum levels of both methylmalonic acid and total homocysteine will be elevated, in about 10% only the homocysteine value is high. In addition, as shown in the accompanying paper in this series [26], antibiotics may normalize the serum methylmalonic acid level without affecting an elevated serum total homocysteine. Since an isolated elevation in total homocysteine is characteristic of folate deficiency [24], a therapeutic trial with a single vitamin, even in pharmacologic doses, may be useful in such patients, since our findings (Figs. 7, 8; Tables I, II) indicate that elevated metabolite levels will only fall to normal when therapy with the vitamin in which the patient is deficient is given.

Ex. 1050, Allen at 97; Ex. 1075, Schiff Reply, ¶68.

Niyikiza and Pretreatment Homocysteine Levels ≥ 10μM

So that suggests that there might be something about having a higher level than 10 that was related to why people were getting toxicity from pemetrexed, and that's what a POSA would have taken out of that set of studies.

Ex. 1086, Zeisel Dep. 29:7-12; Ex. 1075, Schiff Reply ¶¶71-72.

Q For instance, it was common in 1999 to treat high homocysteine with a combination of folate, B6 and B12; correct?

A Very high folate, yes. You know, 30s and 15s would have been treatable at that point.

Q 30s and 15?

A I mean 30 micromolar folate -- I mean homocysteine might have prompted a physician to undergo a treatment.

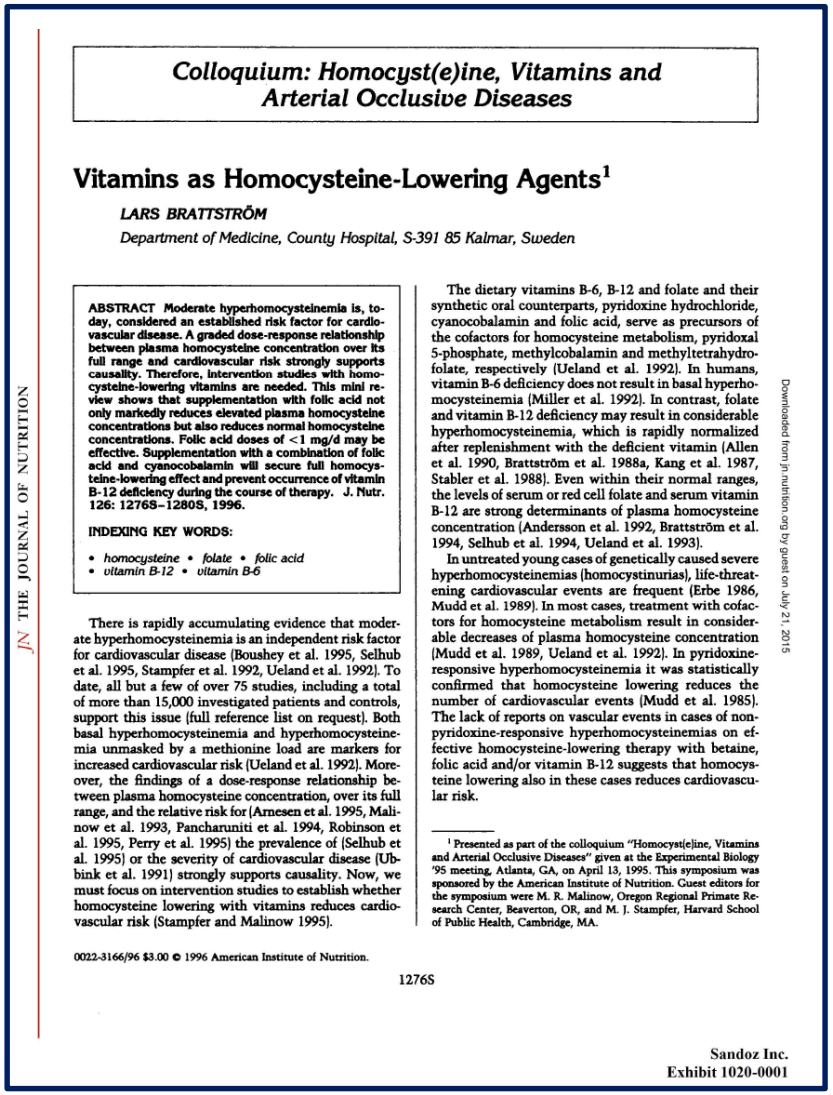
Ex. 1086, Zeisel Dep. 35:18-36:3; Ex. 1075, Schiff Reply ¶71.

Homocysteine and Methylmalonic Acid (MMA)

80. The fact that Niyikiza II reports that "[n]o correlation between toxicity (CTC Grades as defined above) and the remaining pre-specified predictors was seen" does not change my opinion. Ex. 1016, Niyikiza II at 2139. A person of ordinary skill would have been motivated to add vitamin B_{12} even in the absence of this direct correlation between pemetrexed toxicity because of the nature of the statistical analysis reported by the Niyikiza abstracts. The person of ordinary skill would understand that two very highly correlated variables such as methylmalonic acid (MMA) levels and homocysteine may not be discerned as separate variables correlated with the outcome of pemetrexed toxicity. Therefore, the person of ordinary skill would have understood that a correlation between an elevated MMA (indicative of vitamin B_{12} deficiency) and pemetrexed toxicity could in fact exist but may not have been observed because homocysteine and MMA are themselves highly correlated to one another.

Ex. 1004, Schiff ¶80; Ex. 1075, Schiff Reply, ¶66; *see also* Ex. 2120, Chabner Decl. ¶122 .

Prior Art: Brattström (Ex. 1020)



Colloquium: Homocyst(e)ine, Vitamins and Arterial Occlusive Diseases

Vitamins as Homocysteine-Lowering Agents[1]

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ABSTRACT **Moderate hyperhomocysteinemia is, today, considered an established risk factor for cardiovascular disease. A graded dose-response relationship between plasma homocysteine concentration over its full range and cardiovascular risk strongly supports causality. Therefore, intervention studies with homocysteine-lowering vitamins are needed. This mini review shows that supplementation with folic acid not only markedly reduces elevated plasma homocysteine concentrations but also reduces normal homocysteine concentrations. Folic acid doses of <1 mg/d may be effective. Supplementation with a combination of folic acid and cyanocobalamin will secure full homocysteine-lowering effect and prevent occurrence of vitamin B-12 deficiency during the course of therapy. J. Nutr. 126: 1276S–1280S, 1996.**

INDEXING KEY WORDS:

- *homocysteine* • *folate* • *folic acid*
- *vitamin B-12* • *vitamin B-6*

There is rapidly accumulating evidence that moderate hyperhomocysteinemia is an independent risk factor for cardiovascular disease (Boushey et al. 1995, Selhub et al. 1995, Stampfer et al. 1992, Ueland et al. 1992). To date, all but a few of over 75 studies, including a total of more than 15,000 investigated patients and controls, support this issue (full reference list on request). Both basal hyperhomocysteinemia and hyperhomocysteinemia unmasked by a methionine load are markers for increased cardiovascular risk (Ueland et al. 1992). Moreover, the findings of a dose-response relationship between plasma homocysteine concentration, over its full range, and the relative risk for (Arnesen et al. 1995, Malinow et al. 1993, Pancharuniti et al. 1994, Robinson et al. 1995, Perry et al. 1995) the prevalence of (Selhub et al. 1995) or the severity of cardiovascular disease (Ubbink et al. 1991) strongly supports causality. Now, we must focus on intervention studies to establish whether homocysteine lowering with vitamins reduces cardiovascular risk (Stampfer and Malinow 1995).

The dietary vitamins B-6, B-12 and folate and their synthetic oral counterparts, pyridoxine hydrochloride, cyanocobalamin and folic acid, serve as precursors of the cofactors for homocysteine metabolism, pyridoxal 5-phosphate, methylcobalamin and methyltetrahydrofolate, respectively (Ueland et al. 1992). In humans, vitamin B-6 deficiency does not result in basal hyperhomocysteinemia (Miller et al. 1992). In contrast, folate and vitamin B-12 deficiency may result in considerable hyperhomocysteinemia, which is rapidly normalized after replenishment with the deficient vitamin (Allen et al. 1990, Brattström et al. 1988a, Kang et al. 1987, Stabler et al. 1988). Even within their normal ranges, the levels of serum or red cell folate and serum vitamin B-12 are strong determinants of plasma homocysteine concentration (Andersson et al. 1992, Brattström et al. 1994, Selhub et al. 1994, Ueland et al. 1993).

In untreated young cases of genetically caused severe hyperhomocysteinemias (homocystinurias), life-threatening cardiovascular events are frequent (Erbe 1986, Mudd et al. 1989). In most cases, treatment with cofactors for homocysteine metabolism result in considerable decreases of plasma homocysteine concentration (Mudd et al. 1989, Ueland et al. 1992). In pyridoxine-responsive hyperhomocysteinemia it was statistically confirmed that homocysteine lowering reduces the number of cardiovascular events (Mudd et al. 1985). The lack of reports on vascular events in cases of non-pyridoxine-responsive hyperhomocysteinemias on effective homocysteine-lowering therapy with betaine, folic acid and/or vitamin B-12 suggests that homocysteine lowering also in these cases reduces cardiovascular risk.

[1] Presented as part of the colloquium "Homocyst(e)ine, Vitamins and Arterial Occlusive Diseases" given at the Experimental Biology '95 meeting, Atlanta, GA, on April 13, 1995. This symposium was sponsored by the American Institute of Nutrition. Guest editors for the symposium were M. R. Malinow, Oregon Regional Primate Research Center, Beaverton, OR, and M. J. Stampfer, Harvard School of Public Health, Cambridge, MA.

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Hyperhomocysteinemia due to vitamin B-12 deficiency does not respond to folic acid therapy (Allen et al. 1990). It is likely, that even in subjects with low normal vitamin B-12 concentrations full response to folic acid cannot be achieved unless vitamin B-12 is given concomitantly (Landgren et al. 1995). This view is supported by recent studies by Ubbink et al. (1993a, 1993b, 1994). It was shown that men with moderate

Ex. 1020, Brattström at 1277S; Ex. 1004, Schiff Decl. ¶76.

Prior Art: Brönstrup (Ex. 1040)

Effects of folic acid and combinations of folic acid on plasma homocysteine concentrations in healthy

Anja Brönstrup, Monika Hages, Reinhild Prinz Langenohl, and Klaus Pietrzik

ABSTRACT

Background: Elevated plasma homocysteine concentrations are considered to be a risk factor for vascular disease and fetal malformations such as neural tube defects. Recent studies have shown that plasma homocysteine can be lowered by folic acid in amounts corresponding to 1–2 times the recommended dietary allowance. Preliminary evidence indicates that vitamin B-12 may be beneficial when included in supplements or in a food-fortification regimen together with folic acid.

Objective: We aimed to compare the homocysteine-lowering potential of a folic acid supplement with that of 2 supplements containing different doses of vitamin B-12 in addition to folic acid.

Design: Female volunteers of childbearing age (n = 150) received a placebo for 4 wk followed by a 4-wk treatment with either 400 μg folic acid, 400 μg folic acid + 6 μg vitamin B-12, or 400 μg folic acid + 400 μg vitamin B-12.

Results: Significant reductions ($P < 0.001$) in plasma homocysteine were observed in all groups receiving vitamin treatment. The effect observed with the combination of folic acid + 400 μg vitamin B-12 (total homocysteine, −18%) was significantly larger than that with a supplement containing folic acid alone (total homocysteine, −11%) ($P < 0.05$). Folic acid in combination with a low vitamin B-12 dose (6 μg) affected homocysteine as well (−15%).

Conclusions: These results suggest that the addition of vitamin B-12 to folic acid supplements or enriched foods maximizes the reduction of homocysteine and may thus increase the benefits of the proposed measures in the prevention of vascular disease and neural tube defects. *Am J Clin Nutr* 1998;68:1104–10.

KEY WORDS Folic acid, vitamin B-12, supplementation, homocysteine, neural tube defect, cardiovascular disease, women

INTRODUCTION

Homocysteine is being scrutinized as independent risk factor for coronary, cerebral, and peripheral vascular diseases. Most case-control studies and several, though not all, prospective studies have confirmed such an association over a wide range of plasma total homocysteine (tHcy) concentrations (1–4).

In the absence of vitamin B-6 or vitamin B-12 deficiency or genetic defects in non-folate-dependent enzymes, folic acid intervention lowers plasma tHcy concentrations. This has been

1104

ABSTRACT

Background: Elevated plasma homocysteine concentrations are considered to be a risk factor for vascular disease and fetal malformations such as neural tube defects. Recent studies have shown that plasma homocysteine can be lowered by folic acid in amounts corresponding to 1–2 times the recommended dietary allowance. Preliminary evidence indicates that vitamin B-12 may be beneficial when included in supplements or in a food-fortification regimen together with folic acid.

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In this study, vitamin B-12 supplementation increased the tHcy-lowering potential of folic acid; this was especially obvious when vitamin B-12 was given in pharmacologic amounts (400 μg). In subgroup analyses, the extent of the tHcy reduction was significantly higher with the addition of increasing doses of vitamin B-12 in women with a plasma folate concentration >20 nmol/L. Because folate and vitamin B-12 have a synergistic function as cofactors of methionine synthase, sufficiency of both seems to be important to increase enzyme activity, whereas a higher availability of only one cofactor, especially in subjects with an already good supply of this cofactor, might lead to only a limited increase in enzyme activity.

Ex. 1040, Brönstrup at abstract, 1109; Ex. 1004, Schiff Decl. ¶78.

Prior Art: Ubbink I (Ex. 1039)

Human and Clinical Nutrition

Vitamin Requirements for the Treatment of Hyperhomocysteinemia in Humans[1,2]

JOHAN B. UBBINK,[3] W. J. HAYWARD VERMAAK, ANNATJIE VAN DER MERWE, PIET J. BECKER,* RHENA DELPORT AND HENDRIK C. POTGIETER

*Department of Chemical Pathology, Faculty of Medicine, University of Pretoria, 0001 Pretoria, South Africa and *Institute for Biostatistics, Medical Research Council, Pretoria, South Africa*

ABSTRACT We have previously shown that a modest vitamin supplement containing folic acid, vitamin B-12 and vitamin B-6 is effective in reducing elevated plasma homocysteine concentrations. The effect of supplementation of the individual vitamins on moderate hyperhomocysteinemia has now been investigated in a placebo-controlled study. One hundred men with hyperhomocysteinemia were randomly assigned to five groups and treated with a daily dose of placebo, folic acid (0.65 mg), vitamin B-12 (0.4 mg), vitamin B-6 (10 mg) or a combination of the three vitamins for 6 wk. Folic acid supplementation reduced plasma homocysteine concentrations by 41.7% ($P < 0.001$), whereas the daily vitamin B-12 supplement lowered homocysteine concentrations by 14.8% ($P < 0.01$). The daily pyridoxine dose did not reduce significantly plasma homocysteine concentrations. The combination of the three vitamins reduced circulating homocysteine concentrations by 49.8%, which was not significantly different ($P = 0.48$) from the reduction achieved by folate supplementation alone. Our results indicate that folate deficiency may be an important cause of hyperhomocysteinemia in the general population. J. Nutr. 124: 1927–1933, 1994.

INDEXING KEY WORDS:

- *humans* • *homocysteine* • *folate*
- *pyridoxine* • *vitamin B-12*

Patients with premature vascular disorders often have elevated circulating total homocysteine concentrations. Several retrospective studies have linked mild hyperhomocysteinemia to coronary heart disease (Genest et al. 1990, Israelsson et al. 1988, Ubbink et al. 1991a) and cerebral (Brattström et al. 1984, Coull et al. 1990) and peripheral vascular diseases (Malinow et al. 1989, Taylor et al. 1991). Prospective data from the Physicians Health Study also indicate that moderate hyperhomocysteinemia is a risk factor for premature vascular disorders (Stampfer et al. 1992). Participants in the above-mentioned study who subsequently developed myocardial infarction had had significantly higher baseline plasma total homocysteine concentrations when compared with controls matched for age and smoking habits.

Clinical observations support epidemiological findings that elevated plasma homocysteine concentrations are involved in the pathogenesis of atherosclerosis. Taylor et al. (1991) found that the progression of peripheral vascular disease, as assessed in a vascular laboratory, was more common in patients with hyperhomocysteinemia than in patients with normal plasma homocysteine concentrations. Similarly, clinical progression of coronary heart disease, based on new occurrence of angina pectoris, myocardial infarction or congestive heart failure, occurred at a higher rate in patients with hyperhomocysteinemia (Taylor et al. 1991). Malinow et al. (1993) measured the thickness of the intimal-medial carotid walls in individuals free of clinical atherosclerotic disease and found significantly elevated plasma homocysteine concentrations in subjects with thickened intimal-medial carotid walls. Based on the assumption that carotid arterial wall thickening reflects atherosclerosis, the results from Malinow and co-workers suggest involvement of homocysteine in atherosclerotic plaque formation.

The mechanisms by which homocysteine may promote atherogenesis include vascular endothelial

[1]Supported by the Atherosclerosis Risk Factor Research Programme and Vesta Medicines Pty Ltd.
[2]The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.
[3]To whom correspondence and reprint requests should be addressed.

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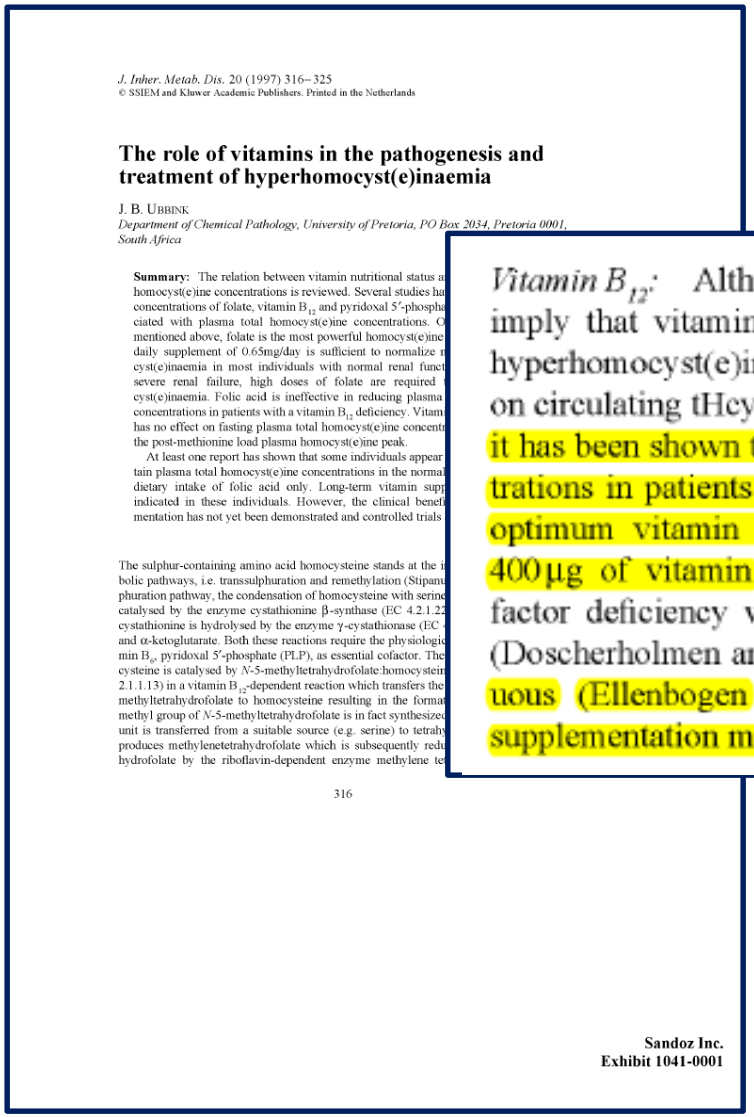
Folic acid supplementation in patients with a chronic vitamin B-12 deficiency may eventually result in neuropathy due to failure to recognize the vitamin B-12 deficiency (Beck 1991). Moreover, Allen et al. (1990) have recently shown that folate supplementation will not correct hyperhomocysteinemia that is primarily the result of a vitamin B-12 deficiency. It is therefore essential that vitamin B-12 and folate be combined to treat hyperhomocysteinemia. Furthermore, it is also important that the cyanocobalamin content of the vitamin supplement should be sufficient to allow adequate absorption of vitamin B-12 from the gastrointestinal tract, even in patients with pernicious anemia. Large oral doses of vitamin B-12 can be used to treat pernicious anemia, because a very small portion of the cobalamin will be absorbed even in the absence of intrinsic factor (Doscherholmen et al. 1957). We used a daily vitamin B-12 supplement of 400 μg (200 × RDA), because it has been calculated that up to 1% of an oral 400-μg vitamin B-12 dose may be absorbed in subjects with pernicious anemia (Ellenbogen and Cooper 1991). This implies that even in cases with intrinsic factor deficiency, ~4 μg (2× RDA) will be absorbed from a daily 400-μg vitamin B-12 dose. We therefore suggest that effective treatment of hyperhomocysteinemia should include at least vitamin B-12 and folic acid supplementation; the vitamin B-12 supplement should be sufficient to satisfy the requirements of patients suffering from intrinsic factor deficiency.

Ex. 1039, Ubbink I, 1931 ; Schiff Decl. ¶77.

Prior Art: Ubbink II (Ex. 1041)



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The role of vitamins in the pathogenesis and treatment of hyperhomocyst(e)inaemia

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Summary: The relation between vitamin nutritional status a
homocyst(e)ine concentrations is reviewed. Several studies ha
concentrations of folate, vitamin B_{12} and pyridoxal 5′-phospha
ciated with plasma total homocyst(e)ine concentrations. O
mentioned above, folate is the most powerful homocyst(e)ine
daily supplement of 0.65mg/day is sufficient to normalize
cyst(e)inaemia in most individuals with normal renal funct
severe renal failure, high doses of folate are required
cyst(e)inaemia. Folic acid is ineffective in reducing plasma
concentrations in patients with a vitamin B_{12} deficiency. Vitam
has no effect on fasting plasma total homocyst(e)ine concentr
the post-methionine load plasma homocyst(e)ine peak.
At least one report has shown that some individuals appear
tain plasma total homocyst(e)ine concentrations in the norma
dietary intake of folic acid only. Long-term vitamin supp
indicated in these individuals. However, the clinical benef
mentation has not yet been demonstrated and controlled trials

The sulphur-containing amino acid homocysteine stands at the i
bolic pathways, i.e. transsulphuration and remethylation (Stipanu
phuration pathway, the condensation of homocysteine with serine
catalysed by the enzyme cystathionine β-synthase (EC 4.2.1.22
cystathionine is hydrolysed by the enzyme γ-cystathionase (EC
and α-ketoglutarate. Both these reactions require the physiologic
min B_6, pyridoxal 5′-phosphate (PLP), as essential cofactor. The
cysteine is catalysed by *N*-5-methyltetrahydrofolate:homocystein
2.1.1.13) in a vitamin B_{12}-dependent reaction which transfers the
methyltetrahydrofolate to homocysteine resulting in the forma
methyl group of *N*-5-methyltetrahydrofolate is in fact synthesized
unit is transferred from a suitable source (e.g. serine) to tetrahy
produces methylenetetrahydrofolate which is subsequently redu
hydrofolate by the riboflavin-dependent enzyme methylene te

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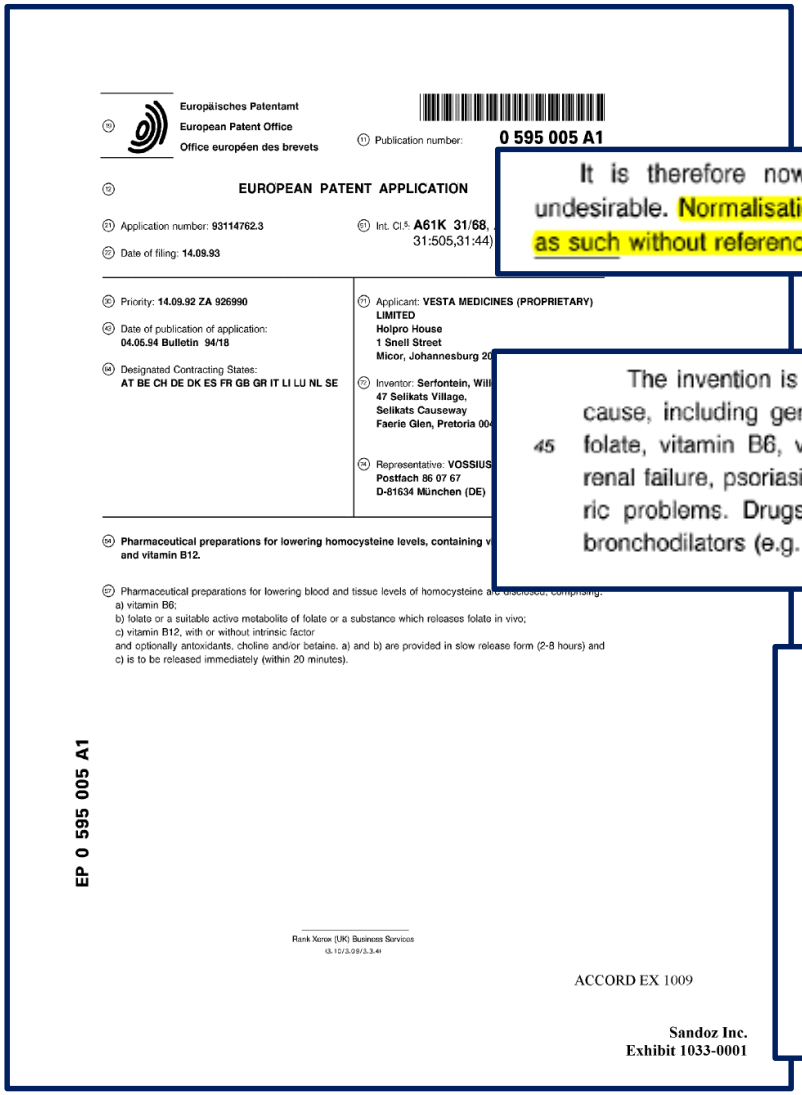
Vitamin B_{12}: Although folic acid is the most powerful tHcy-lowering agent, this does not imply that vitamin B_{12} and vitamin B_6 may be omitted in the treatment of moderate hyperhomocyst(e)inemia. Vitamin B_{12} supplementation has a small, but significant effect on circulating tHcy concentrations (Ubbink et al 1994; Rasmussen et al 1996). Moreover, it has been shown that folic acid supplementation is ineffective in reducing tHcy concentrations in patients with a vitamin B_{12} deficiency (Allen et al 1990). In my opinion, the optimum vitamin supplement to treat hyperhomocyst(e)naemia will contain at least 400 µg of vitamin B_{12} per day. At this high daily dose, even patients with intrinsic factor deficiency will absorb a sufficient amount of vitamin B_{12} by passive diffusion (Doscherholmen and Hagen 1957). Vitamin B_{12} supplementation at high doses is innocuous (Ellenbogen and Cooper 1991) and will eliminate the risk that folic acid supplementation may mask an underlying vitamin B_{12} deficiency.

Ex. 1041, Ubbink II, 321 ; Schiff Decl. ¶77.

Prior Art: European Patent Application 0 595 005 (EP005) (Ex. 1033)



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 595 005 A1**

(19) **EUROPEAN PATENT APPLICATION**

(21) Application number: **93114762.3**

(51) Int. Cl.5: **A61K 31/68**, 31:505,31:44)

(22) Date of filing: **14.09.93**

(30) Priority: **14.09.92 ZA 926990**

(43) Date of publication of application:
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(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

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(54) **Pharmaceutical preparations for lowering homocysteine levels, containing v and vitamin B12.**

(57) Pharmaceutical preparations for lowering blood and tissue levels of homocysteine are disclosed, comprising:
a) vitamin B6;
b) folate or a suitable active metabolite of folate or a substance which releases folate in vivo;
c) vitamin B12, with or without intrinsic factor
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EP 0 595 005 A1

Rank Xerox (UK) Business Services
(3.10/3.09/3.3.4)

ACCORD EX 1009

Sandoz Inc.
Exhibit 1033-0001

It is therefore now accepted in the art that elevated blood levels of homocysteine are highly undesirable. Normalisation of such elevated levels of homocysteine therefore constitutes a therapeutic goal as such without reference to any specific disease entity, possibly causally related to such elevated levels.

Ex. 1033 EP005, 3:7-9; Ex. 1004, Schiff ¶79; Ex. 1075, Schiff Reply, ¶¶78-82.

The invention is applicable to the lowering of total homocysteine blood levels if elevated by any known cause, including genetic causes (e.g. enzyme polymorphism) diets, drugs or depressed activity levels of folate, vitamin B6, vitamin B12 or any combination of these due to whatever cause, pregnancy, chronic renal failure, psoriasis, occlusive vascular disease, chronic liver disease, homocysteine-associated psychiatric problems. Drugs which induce elevated homocysteine levels include anticonvulsant drugs, xanthine bronchodilators (e.g. theophylline), methotrexate, nitrous oxide, and many others.

Ex. 1033 EP005, 4:43-48; Ex. 1075, Schiff Reply, ¶¶78-82.

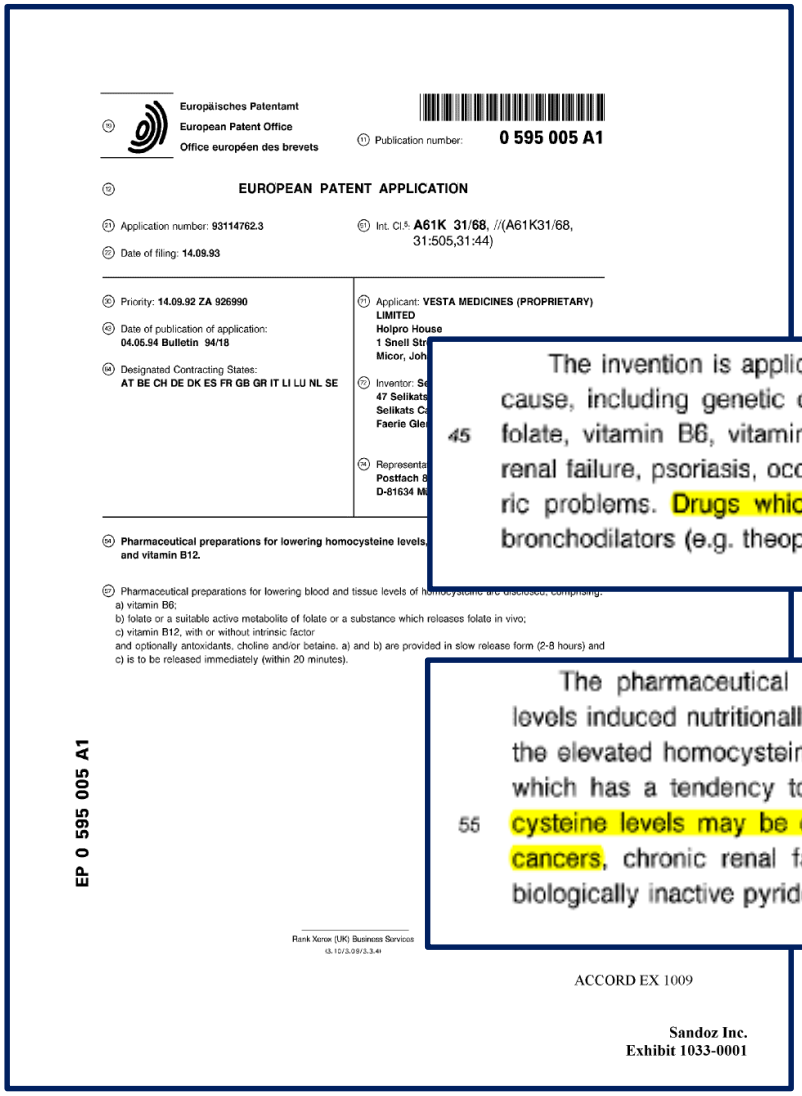
24 Q. All right. So one of ordinary skill in
25 the art in June of 1999, if they were interested
1 in controlling blood homocysteine levels, what
2 this reference tells them that you can do that
3 with a combination of Vitamin B12 and folic acid.
4 Correct?

. . .

7 A. It says that. That's -- that's true.

Ex. 1074, Chabner Dep. Tr. 185:24-185:7.; Ex. 1075, Schiff Reply, ¶78

Prior Art: European Patent Application 0 595 005 (EP005) (Ex. 1033)



(19) Europäisches Patentamt
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(54) **Pharmaceutical preparations for lowering homocysteine levels and vitamin B12.**

(57) Pharmaceutical preparations for lowering blood and tissue levels of homocysteine are disclosed, comprising:
a) vitamin B6;
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c) vitamin B12, with or without intrinsic factor
and optionally antioxidants, choline and/or betaine. a) and b) are provided in slow release form (2-8 hours) and c) is to be released immediately (within 20 minutes).

EP 0 595 005 A1

Rank Xerox (UK) Business Services
(3.10/3.09/3.3.4)

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Sandoz Inc.
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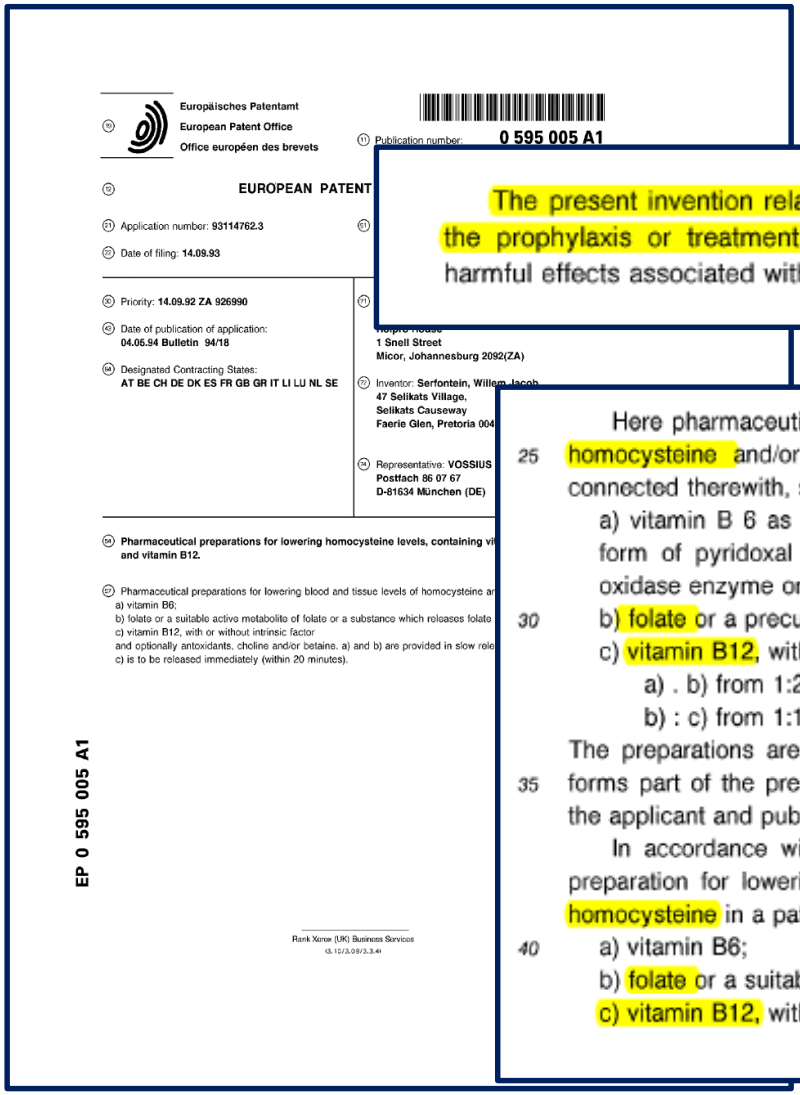
Ex. 1033 EP005, 4:43-48; Ex. 1075, Schiff Reply, ¶¶78-82.

The pharmaceutical compositions are not only to be used in the treatment of raised homocysteine levels induced nutritionally, genetically or as a result of a variety of diseases, but also in those cases where the elevated homocysteine levels are drug induced or in combination with a B6 or folate antagonistic drug, which has a tendency to raise homocysteine levels. Examples of other situations in which blood homocysteine levels may be elevated are the following: post-menopausal women, liver failure, leukemia, other cancers, chronic renal failure. Slow-release formulation of PL prevents excessive liver oxidation to the biologically inactive pyridoxic acid.

Ex. 1033, EP005, 9:51-57.

Prior Art: European Patent Application 0 595 005 (EP005) (Ex. 1033)



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EP 0 595 005 A1

Rank Xerox (UK) Business Services

The present invention relates to pharmaceutical preparations for lowering levels of homocysteine or for the prophylaxis or treatment of elevated levels of homocysteine in patients and for counteracting the harmful effects associated with homocysteine.

Here pharmaceutical and dietary preparations are disclosed for the treatment or prophylaxis of elevated homocysteine and/or methionine levels in the blood of human infants and pathological disturbances connected therewith, said preparation comprising in combination:-

a) vitamin B 6 as such or in the form of a pharmaceutically acceptable acid salt, at least in part in the form of pyridoxal (PL) or a compound which in vivo readily releases PL without the intervention of oxidase enzyme or oxygen.

b) folate or a precursor of folate which releases folate in vivo, and

c) vitamin B12, with or without intrinsic factor, in the following ratios:-

a) . b) from 1:25 to 10 000 : 1

b) : c) from 1:1 to 50 000 : 1

The preparations are to be incorporated in infant bone feed mixes. That disclosure, by cross-reference, forms part of the present disclosure. The same applies to the contents of a study performed on behalf of the applicant and published after the priority date hereof in Am.J.Clinical Nutrition (1993), 57, pp 47-53.

In accordance with the invention there is provided the use in the manufacture of a pharmaceutical preparation for lowering levels of homocysteine or for the prophylaxis or treatment of elevated levels of homocysteine in a patient of a combination which comprises

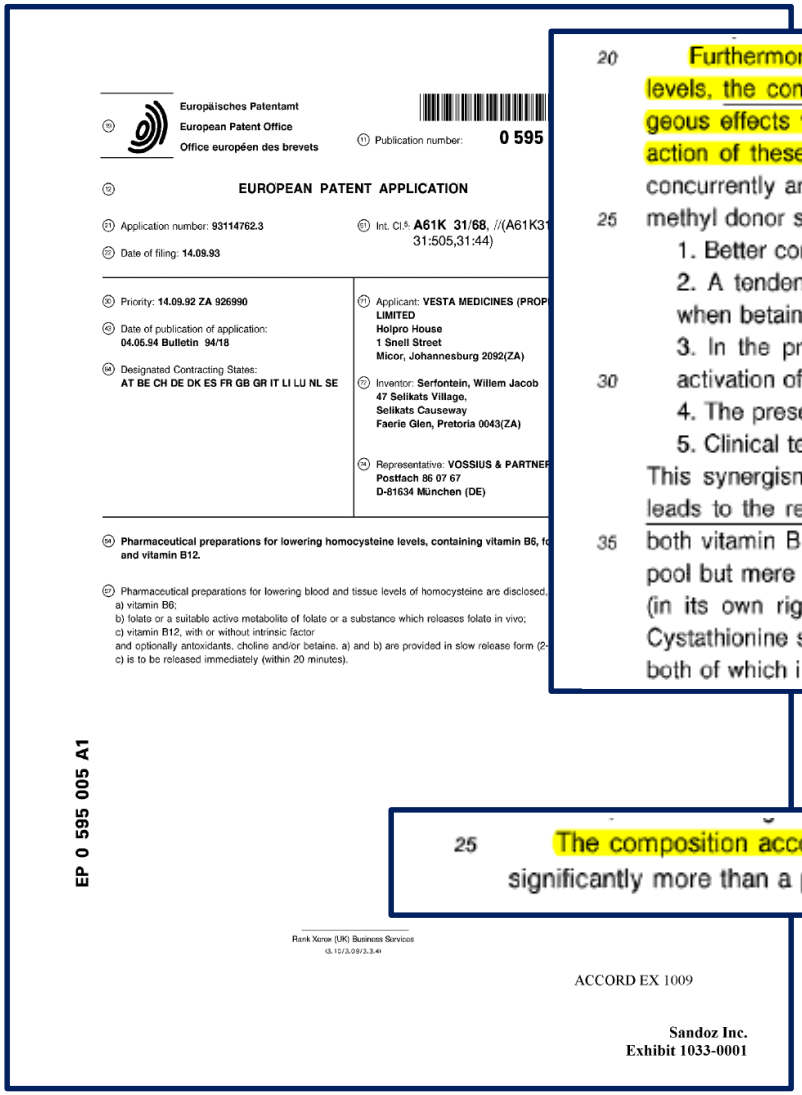
a) vitamin B6;

b) folate or a suitable active metabolite of folate or a substance which releases folate in vivo;

c) vitamin B12, with or without intrinsic factor.

Ex. 1033 EP005, 2:1-3, 4:24-42; Ex. 1004, Schiff Decl. ¶79; Ex. 1075, Schiff Reply, ¶¶78-82.

Prior Art: European Patent Application 0 595 005 (EP005) (Ex. 1033)



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(11) Publication number: 0 595

EUROPEAN PATENT APPLICATION

(21) Application number: 93114762.3
(22) Date of filing: 14.09.93
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(30) Priority: 14.09.92 ZA 926990
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(74) Representative: VOSSIUS & PARTNER
Postfach 86 07 67
D-81634 München (DE)

(54) Pharmaceutical preparations for lowering homocysteine levels, containing vitamin B6, fo and vitamin B12.

(57) Pharmaceutical preparations for lowering blood and tissue levels of homocysteine are disclosed.
a) vitamin B6;
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EP 0 595 005 A1

Rank Xerox (UK) Business Services
(3.10/3.09/3.3.4)

ACCORD EX 1009

Sandoz Inc.
Exhibit 1033-0001

Furthermore, applicant has surprisingly found that for purposes of controlling blood homocysteine levels, the combination in accordance with the invention of PL, folate and vitamin B12 produces advantageous effects which go substantially beyond what might be expected from a simple additive effect of the action of these drugs. Thus, an unexpected synergism exists when vitamin B12, folate and PL are given concurrently and this effect can be even greater when the vitamins are given in conjunction with a biological methyl donor such as choline or betaine. This synergism is evidenced by:

1. Better control of blood homocysteine levels at lower dosage levels of each.
2. A tendency to restore to normality distorted blood amino acid patterns which are sometimes seen when betaine is given alone.
3. In the presence of both folate and PL, methionine levels do not rise as much after betaine due to activation of alternative metabolic pathways.
4. The presence of PL limits damage to structural proteins, especially in the vascular bed.
5. Clinical tests. (See examples)

This synergism may further be appreciated from the fact that PL stimulates a process which ultimately leads to the reduction of the methionine pool (through conversion of homocysteine into cysteine) whereas both vitamin B12 and folate stimulate processes which do not lead to a reduction of the body's methionine pool but mere recycling. The resultant methionine remains available for reconversion into homocysteine. PL (in its own right and distinct from PLP) has co-enzyme activity for the enzyme cystathionine synthase. Cystathionine synthase activity can be stimulated in a dose dependent manner by intracellular PLP and PL, both of which increase after administration of PL.

Ex. 1033 EP005, 11:20-25; Paper 49,Sandoz Reply, p. 14

The composition according to the invention is nearly twice as effective as folate alone. This indicates a significantly more than a purely additive effect of the three component combination (synergism).

Ex. 1033 EP005, 18:25-26

Prior Art: Folic Acid and Vitamin B_{12} With Antifolates Prior to June 1999

The New England Journal of Medicine

Volume 238 JUNE 3, 1948 Number 23

TEMPORARY REMISSIONS IN ACUTE LEUKEMIA IN CHILDREN PRODUCED BY FOLIC ACID ANTAGONIST, 4-AMINOPTEROYL-GLUTAMIC ACID (AMINOPTERIN)*

Sidney Farber, M.D.,† Louis K. Diamond, M.D.,‡ Robert D. Mercer, M.D.,§ Robert F. Sylvester, Jr., M.D.,¶ and James A. Wolff, M.D.‖

BOSTON

IT IS the purpose of this paper to record the results of clinical and hematologic studies on 5 children with acute leukemia treated by the intramuscular injection of a synthetic compound, 4-aminopteroylglutamic acid (aminopterin). This substance is an antagonist to folic acid regarding the growth of *Streptococcus faecalis R*.

Sandoz Inc. Exhibit 1009-0001

Farber (1948)

most normal pattern. Toxic effects included stomatitis, with early ulceration. In an attempt to prevent this complication crude liver extract was employed, as were folic acid and folic acid conjugates.

Ex. 1009, Farber at 792; Ex. 1075, Schiff Reply ¶96; Ex. 1091, Stover Reply ¶17

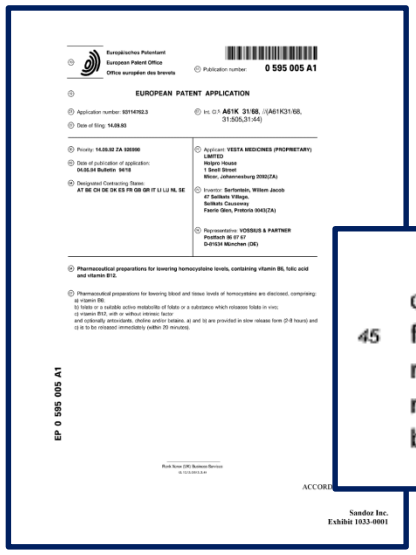
Tisman 1985

POSSIBLE POTENTIATION OF FLUOROPYRIMIDINE ANTI-TUMOR ACTIVITY BY PTEROYLGLUTAMIC ACID (FOLIC ACID) AND CYANOCOBALAMIN (B_{12}). Glenn Tisman, Victoria Flener, Mary E. Jones, Lynette Buck. Whittier, CA. 90601.

Laboratory studies confirmed that both folinic acid and folic acid can potentiate 5FU activity against different tumor cells. Our preliminary clinical work with attempts to potentiate 5FU activity with low doses (3mg) of Leucovorin was unsuccessful (Tisman, et.al., AACR, 19, 1978, 217A). Because folic acid may be the preferred substrate for intracellular conversion to polyglutamates (Perry, J., et.al., 1979), and because reduced folate polyglutamates potentiate the binding of 5FdUMP to thymidylate synthetase, we felt that large doses of folic acid might potentiate 5FU oncolytic effects clinically. Folic acid 200 mg/m^2 plus Cyanocobalamin 10,000mcg (used to enhance intracellular transport of folate) (Herbert, Tisman, et.al., Blood 4:465, 1973) in 200ml. of ½N saline plus 20MEq of sodium bicarbonate was infused I.V. [illegible] first hour of each infusion a [illegible] treatment was repeated weekly. [illegible] ractory to 5FU containing [illegible] f 3 patients had an exacerbation of [illegible] initiation of therapy. All [illegible] e pain within 1 week. CEA [illegible] gic toxicity was not significant [illegible] patients developed diarrhea at the end of 1 and 3 weeks. Red blood cell folate levels after therapy revealed red cell folates (polyglutamates) were 2 to 3 times normal. The above protocol is clearly associated with tumor response to 5FU in 5FU refractory patients.

POSSIBLE POTENTIATION OF FLUOROPYRIMIDINE ANTI-TUMOR ACTIVITY BY PTEROYLGLUTAMIC ACID (FOLIC ACID) AND CYANOCOBALAMIN (B_{12}). Glenn Tisman, Victoria Flener, Mary E. Jones, Lynette Buck. Whittier, CA.

Ex. 1028, Tisman; Ex. 1004, Schiff Decl. ¶89



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Publication number: 0 595 005 A1

EUROPEAN PATENT APPLICATION

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EP 0 595 005 A1

Sandoz Inc. Exhibit 1033-0001

EP 005 (1994)

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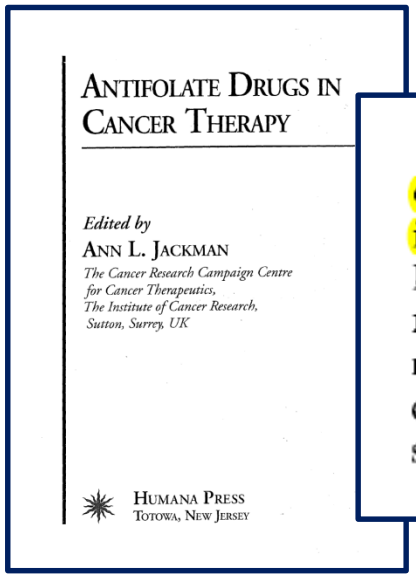
Ex. 1033, EP005, 4:43-48; Ex. 1075, Schiff Reply, ¶¶78-82.

Prior Art: Folic Acid and Vitamin B_{12} and Antifolates before June 1999


ANTIFOLATE DRUGS IN CANCER THERAPY

Edited by
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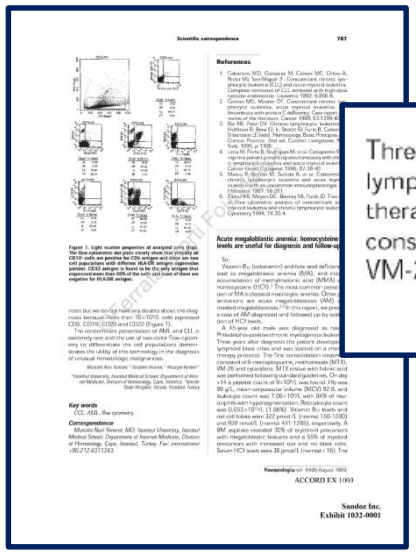
The Cancer Research Campaign Centre for Cancer Therapeutics, The Institute of Cancer Research, Sutton, Surrey, UK

HUMANA PRESS
TOTOWA, NEW JERSEY

Mendelsohn (1999)

The biochemical pathways that utilize folate cofactors also require adequate amounts of vitamins B_{12} and B_6. Thus, the status of all three vitamins in patients may significantly influence the severity of toxicity observed during chemotherapy. R. Allen and his colleagues have established that measuring specific amino acid metabolites, especially homocysteine, N-methyl glycine and others, from these metabolic pathways provides a more sensitive and reliable assessment of patient vitamin status *(23)*. These surrogate indicators of functional folate status are more indicative of deficiencies and more responsive to dietary supplementation.

Ex. 1012, Mendelsohn at 270; Ex. 1004, Schiff Decl. ¶¶49; Ex. 1075, Schiff Reply ¶77


Scientific correspondence 767

References

Acute megaloblastic anemia: homocysteine levels are useful for diagnosis and follow-up

ACCORD EX 1003

Sandoz Inc.
Exhibit 1032-0001

Carrasco (1999)

Three years after diagnosis the patient developed a lymphoid blast crisis and was started on a chemotherapy protocol. The first consolidation treatment consisted of 6-mercaptopurine, methotrexate (MTX), VM-26 and cytarabine. MTX rescue with folinic acid

Serum HCY levels were 38 µmol/L (normal < 16). The patient was diagnosed as having AM and began treatment with folinic acid 12 mg iv in one single dose and folic acid 5 mg/day po for 14 days and parenteral vitamin B_{12} 2 mg/day for 4 consecutive days.

Ex. 1032, Carrasco, 768 ; Ex. 1075, Schiff Reply, ¶103; Paper 2, Pet. at 32.

Prior Art: Tisman (1985)

POSSIBLE POTENTIATION OF FLUOROPYRIMIDINE ANTI-TUMOR ACTIVITY BY PTEROYLGLUTAMIC ACID (FOLIC ACID) AND CYANOCOBALAMIN (B_{12}). Glenn Tisman, Victoria Flener, Mary E. Jones, Lynette Buck. Whittier, CA. 90601.

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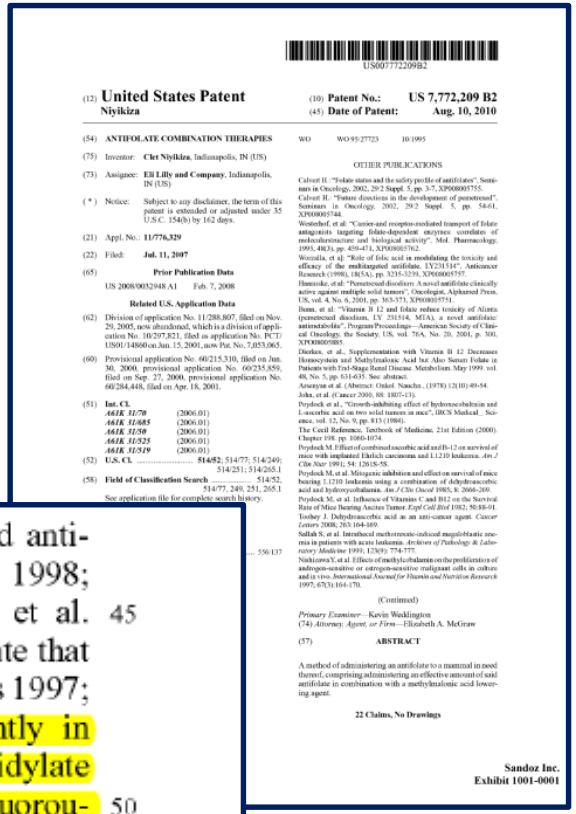
Ex. 1028, Tisman; Ex. 1004, Schiff Decl. ¶89



US007772209B2

(12) **United States Patent**
Niyikiza

(10) **Patent No.: US 7,772,209 B2**
(45) **Date of Patent: Aug. 10, 2010**

(54) **ANTIFOLATE COMBINATION THERAPIES**

(75) Inventor: **Clet Niyikiza**, Indianapolis, IN (US)

(73) Assignee: **Eli Lilly and Company**, Indianapolis, IN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 162 days.

(21) Appl. No.: **11/776,329**

(22) Filed: **Jul. 11, 2007**

(65) **Prior Publication Data**

US 2008/0032948 A1 Feb. 7, 2008

Related U.S. Application Data

(62) Division of application No. 11/288,807, filed on Nov. 29, 2005, now abandoned, which is a division of application No. 10/297,821, filed as application No. PCT/US01/14860 on Jun. 15, 2001, now Pat. No. 7,053,065.

(60) Provisional application No. 60/215,310, filed on Jun. 30, 2000, provisional application No. 60/235,859, filed on Sep. 27, 2000, provisional application No. 60/284,448, filed on Apr. 18, 2001.

(51) **Int. Cl.**
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A61K 31/685 (2006.01)
A61K 31/50 (2006.01)
A61K 31/525 (2006.01)
A61K 31/519 (2006.01)

(52) U.S. Cl. **514/82**; 514/77; 514/249; 514/251; 514/265.1

(58) **Field of Classification Search** 514/52, 514/77, 249, 251, 265.1
See application file for complete search history.

WO WO 95/27723 10/1995

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(Continued)

Primary Examiner—Kevin Weddington
(74) *Attorney, Agent, or Firm*—Elizabeth A. McGraw

(57) **ABSTRACT**

A method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

22 Claims, No Drawings

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Exhibit 1001-0001

tion: Mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). Advan Enzyme Regul, 1998; 38:135-152 and Shih C, Chen V J, Gossett L S, et al. LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. Cancer Res 1997; 57:1116-1123.) Several antifolate drugs are currently in development. Examples of antifolates that have thymidylate synthase inhibiting ("TSI") characteristics include 5-fluorouracil and Tomudex®. An example of an antifolate that has

Ex. 1001, col. 1, ll. 43-51.

Prior Art: Mendelsohn (1999)

ANTIFOLATE DRUGS
CANCER THERAPY

The biochemical pathways that utilize folate cofactors also require adequate amounts of vitamins B_{12} and B_6. Thus, the status of all three vitamins in patients may significantly influence the severity of toxicity observed during chemotherapy. R. Allen and his colleagues have established that measuring specific amino acid metabolites, especially homocysteine, N-methyl glycine and others, from these metabolic pathways provides a more sensitive and reliable assessment of patient vitamin status *(23)*. These surrogate indicators of functional folate status are more indicative of deficiencies and more responsive to dietary supplementation.

Ex. 1012, Mendelsohn at 270; Ex. 1004, Schiff Decl. ¶¶49; Ex. 1075, Schiff Reply ¶77

Table 1
Inhibition of hG
by Lometrexol, 254155, an

Compound No.	*Compound Nam*	
LY249543	lometrexol	
LY235540	diglu	
LY235337	triglu	13.3 ($n = 2$)
LY266978	tetraglu	7.1 ± 2.2 ($n = 4$)
LY235542	pentaglu	5.3 ($n = 2$)
LY254155	thienyl-DDATHF	2.1 ± 0.2 ($n = 5$)
LY314565	diglu	1.2 ($n = 1$)
LY314209	triglu	0.25 ($n = 2$)

The potency of antifolate analogs to inhibit monofunctional human GARFT was assessed spectrophometrically using the Morrison equation, which is appropriate for determining the affinity of "tight-binding" compounds that produce stoichiometric inhibition (*2,4*).

Table 2
Kinetic Constants for Activation of GARFT Inhibitors by FPGS

Compound	K_m (μM)	*Vmax (μmoles/h/mg)*	$k'(V_m/K_m)$
lometrexol	16.4 ± 1.0	977 ± 128	60
LY309887	6.5 ± 1.1	686 ± 116	43
MTA	1.9 ± 0.5	725 ± 95	381

tion of lometrexol and LY309887, determined using hog liver FPGS are summarized in Table 2 *(5)*. Lometrexol and LY309887 had similar K_m values as FPGS substrates. However, lometrexol had a significantly higher V_{max}. The relative efficiencies of substrate utilization by an enzyme can be determined by comparing first-order rate constants, k′ (V_{max}/K_m). The data suggest that desp
strate, which would be more extensive
obtained with the multitargeted antifo
a first-order rate constant of 381, it cl
FPGS substrate. In other experiments,
cubation of lometrexol, LY309887 or
HPLC. At low substrate concentration
all substrates was more extensive and
verted to tetra- and pentaglutamated fo
in which over 70% of each antifolate
servations are consistent with the know
at high intracellular folate concentrati
An important inference from these
late and retain greater amounts of hig
ularly in liver, a known folate depot,
cycling of stored antifolate through t
nomenon of delayed and cumulative

Table 2
Kinetic Constants for Activation of GARFT Inhibitors by FPGS

Compound	K_m (μM)	*Vmax (μmoles/h/mg)*	$k'(V_m/K_m)$
lometrexol	16.4 ± 1.0	977 ± 128	60
LY309887	6.5 ± 1.1	686 ± 116	43
MTA	1.9 ± 0.5	725 ± 95	381

(V_{max}/K_m). The data suggest that despite equal K_m values, lometrexol was a better substrate, which would be more extensively polyglutamated in vivo. For comparison, data obtained with the multitargeted antifolate inhibitor, LY231514 (MTA), is shown. With a first-order rate constant of 381, it clearly had the greatest affinity and efficacy as an FPGS substrate. In other experiments, polyglutamated products formed during a 24-h incubation of lometrexol, LY309887 or MTA with FPGS were separated by quantitative HPLC. At low substrate concentrations, i.e., below the K_m (1 μM), polyglutamation of

Ex. 1012, Mendelsohn at 263; Ex. 1004, Schiff Decl. ¶¶49; Ex. 1075, Schiff Reply ¶77

Prior Art: Carrasco (1999)

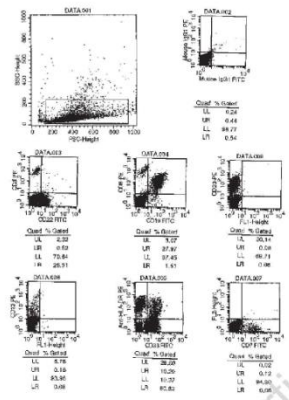

Figure 1. Light scatter properties of analyzed cells (top). The flow cytometric dot plots clearly show that virtually all CD19+ cells are positive for CD5 antigen and there are two cell populations with different HLA-DR antigen expression pattern. CD33 antigen is found to be the only antigen that expressed more than 50% of the cells and most of them are negative for HLA-DR antigen.

nosis but we do not have any doubts about the diagnosis because more then 10×10^9/L cells expressed CD5, CD19, CD20 and CD22 (Figure 1).

The concomitant presentation of AML and CLL is extremely rare and the use of two-color flow cytometry to differentiate the cell populations demonstrates the utility of this technology in the diagnosis of unusual hematologic malignancies.

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Key words
CCL, AML, flow cytometry.

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Acute megaloblastic anemia: homocysteine levels are useful for diagnosis and follow-up

Sir,

Vitamin B_{12} (cobalamin) and folic acid deficiencies lead to megaloblastic anemia (MA), and induce accumulation of methylmalonic acid (MMA) and homocysteine (HCY).[1] The most common presentation of MA is classical macrocytic anemia. Other presentations are acute megaloblastosis (AM) and masked megaloblastosis.[2,3] In this report, we present a case of AM diagnosed and followed up by evaluation of HCY levels.

A 45-year old male was diagnosed as having Philadelphia-positive chronic myelogenous leukemia. Three years after diagnosis the patient developed a lymphoid blast crisis and was started on a chemotherapy protocol. The first consolidation treatment consisted of 6-mercaptopurine, methotrexate (MTX), VM-26 and cytarabine. MTX rescue with folinic acid was performed following standard guidelines. On day +14 a platelet count of 9×10^9/L was found. Hb was 99 g/L, mean corpuscular volume (MCV) 92 fL and leukocyte count was 7.06×10^9/L with 84% of neutrophils with hypersegmentation. Reticulocyte count was 0.053×10^{12}/L (1.66%). Vitamin B_{12} levels and red cell folate were 322 pmol /L (normal 150-1200) and 938 nmol/L (normal 441-1285), respectively. A BM aspirate revealed 30% of erythroid precursors with megaloblastic features and a 55% of myeloid precursors with increased size and no blast cells. Serum HCY levels were 38 µmol/L (normal < 16). The

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Exhibit 1032-0001

Three years after diagnosis the patient developed a lymphoid blast crisis and was started on a chemotherapy protocol. The first consolidation treatment consisted of 6-mercaptopurine, methotrexate (MTX), VM-26 and cytarabine. MTX rescue with folinic acid Serum HCY levels were 38 µmol/L (normal < 16). The patient was diagnosed as having AM and began treatment with folinic acid 12 mg iv in one single dose and folic acid 5 mg/day po for 14 days and parenteral vitamin B_{12} 2 mg/day for 4 consecutive days.

Ex. 1032, Carrasco, 767-768 ; Ex. 1075, Schiff Reply, ¶103; Paper 2, Pet. at 32.

Dr. Chabner's Proposed Alternatives to Folic Acid + Vitamin B_{12}

1. Dose reductions
2. Leucovorin Rescue
3. Granulocyte Colony Stimulating Factor
4. Betaine

Ex. 1075, Schiff Reply, ¶¶22

Dr. Chabner's Alternative #1: Dose Reductions

Q. So one of ordinary skill in the art would understand in June 1999 that these dose reductions --

A. Uh-huh.

Q. -- could have an impact on the efficacy of pemetrexed on the cancer?

A. Right. It could have. As I said, I think they very quickly devised a way of avoiding many of these dose reductions by -- by using dexamethasone. And that's still being done today.

Phase I–Phase II Trial of *N*-Phosphonacetyl-L-Aspartic Acid Given by Intravenous Infusion and 5-Fluorouracil Given by Bolus Injection [1,2]

Charles Erlichman,[3,4] Ross C. Donehower,[5] James L. Speyer,[6] Ray Klecker,[7] *and* Bruce A. Chabner[7]

and FUra could not be administered to him. Our findings suggest that this particular dose schedule for PALA and FUra would result in less than a 25% response rate ($P<0.05$) for patients with melanoma and, therefore, would not be a useful combination for clinical therapy of melanoma.

Ex. 1074, Chabner Dep. 234:11-17; Ex. 1075, Schiff Reply, ¶23; Ex. 1094, Erlichman, 230

Dr. Chabner's Alternative #2: Granulocyte Colony Stimulating Factor

inflammatory agents.) Supportive-care agents, such as colony-stimulating factors, were permitted but could not be substituted for dose reductions required according to protocol. No dose escalations were permitted.

Ex. 1052, Rusthoven, 1195, 1196; Ex. 1075, Schiff Reply ¶¶ 28-31

treatment. Four patients (13.3%) experienced febrile neutropenia and 13 (39%) experienced grade 3 or 4 neutropenia, whereas only one patient (3%) developed grade 4 thrombocytopenia. Nonhematologic toxicity was generally mild or moderate, but 39% of patients developed a grade 3 skin rash. Most other toxicities comprised grade 1 or 2 stomatitis, diarrhea, lethargy, and anorexia. Ten patients stopped protocol therapy because of toxicity.

Ex. 1052, Rusthoven, 1195, 1196; Ex. 1075, Schiff Reply ¶¶ 28-31

Oh, okay. Yeah. Colony stimulating factors were permitted, but could not be substituted for dose reductions required. So the idea is this,

Ex. 1074, Chabner Dep. 234:24-236:7; 267:16-268:1; Ex. 1075, Schiff Reply ¶¶ 28-31

Dr. Chabner's Alternative #3: Leucovorin Rescue

[CANCER RESEARCH 55, 6117–6125, December 15, 1995]

Enhanced Antitumor Activity for the Thymidylate Synthase Inhibitor 1843U89 through Decreased Host Toxicity with Oral Folic Acid

Gary K. Smith,[1,2] Herbert Amyx, Christine M. Boytos,[1] David S. Duch, Robert Ferone, and H. Robert Wilson

Divisions of Cell Biology [G. K. S., D. S. D.], Biochemistry [C. M. B., R. F., H. R. W.], and Toxicology [H. A.], The Wellcome Research Labs, Burroughs Wellcome, Research Triangle Park, North Carolina 27709

only increased the 1843U89 IC_{50} 1- to 3-fold. Thus, the efficacy of 1843U89 in cell culture is less sensitive than Tomudex to reversal by either leucovorin or folic acid, and folic acid is the less effective reversing agent.

weight loss caused by 200 mg/kg 1843U89. A higher dose of 1843U89, 400 mg/kg, was lethal to all mice by day 10 in the absence of folic acid, but in the presence of the protectant, 80% of the animals survived (Fig. 2). Thus, folic acid can protect against the lethal toxicity of 1843U89 in mice as well as dogs.

Ex. 2040, Smith & Amyx, 6119, 6120; Ex. 1075, Schiff Reply ¶¶ 25-27

Q. You were asked a question:

"QUESTION: Dr. Chabner, you agree that all rescue strategies could have a negative impact on efficacy as a chemotherapy agent, right?

"ANSWER: That's correct."

Ex. 1074, Chabner Dep. 281:10-14; Ex. 1075, Schiff Reply, ¶¶ 25-27

Dr. Chabner's Alternative #4: Betaine

Elevation of Homocysteine and Excitatory Amino Acid Neurotransmitters in the CSF of Children Who Receive Methotrexate for the Treatment of Cancer

By Charles T. Quinn, James C. Griener, Teodoro Bottiglieri, Keith Hyland, Arleen Farrow, and Barton A. Kamen

Purpose: Folate deficiency, either by diet or drug, increases plasma homocysteine (Hcy). Hcy damages cerebrovascular endothelium, and hyperhomocysteinemia is a risk factor for stroke. Hcy is metabolized to excitatory amino acid (EAA) neurotransmitters, such as homocysteic acid (HCA) and cysteine sulfinic acid (CSA), which may cause seizures and excitotoxic neuronal death. We postulated that excess Hcy and EAA neurotransmitters may partly mediate methotrexate (MTX)-associated neurotoxicity.

Patients and Methods: In this retrospective analysis, we used high-performance liquid chromatography (HPLC) to measure Hcy, HCA, and CSA in CSF from two groups of children: (1) a control group of patients with no MTX exposure, and (2) a treatment group of patients who had received MTX no more than 7 days before a scheduled lumbar puncture.

Results: The treatment group had a significantly ($P = .0255$) greater concentration of Hcy in CSF (0.814 μmol/L ± 0.215 [mean ± SEM], n = 23) than the control group (0.210 μmol/L ± 0.028, n = 34). HCA and CSA were not detected in CSF from control patients (n = 29); however, MTX caused marked accumulation of CSF HCA (119.1 μmol/L ± 32.0, n = 16) and CSA (28.4 μmol/L ± 7.7, n = 16) in the treatment group. Patients with neurologic toxicity at the time of lumbar puncture had many of the highest concentrations of Hcy, HCA, and CSA.

Conclusion: These data support our hypothesis that MTX-associated neurotoxicity may be mediated by Hcy and excitotoxic neurotransmitters.

J Clin Oncol 15:2800-2806. © 1997 by American Society of Clinical Oncology.

Ex. 2033, Quinn

Q. And Quinn is talking about using betaine post treatment to rescue patients?

A. I don't think he ever used it. He didn't.

Ex. 1074, Chabner Dep. 278:4-14; Ex. 1075, Schiff Reply, ¶ 33

34. Moreover, betaine is not a required nutrient like folate for life. Treating folate- or vitamin-B12 deficiency in a way that ignores the deficiencies of these essential nutrients places the patients at risk for related diseases including cancer and neuropathies. There are also certain adverse effects of betaine administration that are disrupting and unpleasant as it is known to cause extreme body odor.

Ex. 1091 Stover Reply,¶¶ 32-34

A I don't understand the interpretation of "baseline." But if you meant what a -- how many -- what portion of the population had low dietary betaine and its precursor, intake, we didn't know that until more recently.

Ex. 1086, Zeisel Dep. 285:23-286:7; Ex. 1091, Stover Reply, ¶ 32

Prior Art: Arsenyan (Ex. 1023)

INFLUENCE OF METHYLCOBALAMIN ON THE ANTINEOPLASTIC ACTIVITY OF METHOTREXATE

F. G. Arsenyan, N. V. Myasishcheva, Z. P. Sof'ina, M. O. Raushenbakh, I. P. Rudakova, E. G. Chauser, and A. M. Yurkevich

UDC 615,277.3.015.2:615.355:577. 152.611'.133

One of the possible ways of increasing the selectivity of the action of chemotherapeutic substa tumor cells is the combined use of preparations, taking the peculiarities of the mechanism of their account. A new trend in this field is the use of cobalamin derivatives in combination with definite an plastic preparations.

The special significance of methylcobalamin was first noted in the case of impaired cobalamin olism in leukemia patients. An analysis of the functional activity of cobalamin coenzymes in the or in comparison with the effectiveness of combined cytostatic therapy, has shown that the clinical cou process in acute leukemia with an increased content of hydroxy- and methylcobalamins in the blood favorable [1]. The results obtained were evidence of the important role of methylcobalamin in meta processes as a coenzyme of methionine synthetase (EC 2.1.1.13)—a key link in the control of the syn action of cobalamins in compounds of folic acid in processes of cell proliferation [1-2].

A study of the morphofunctional state of the hemopoietic system of animals under conditions o cobalamin metabolism in the organism confirmed the fact that at a high concentration of cobalamin the rate of proliferation of cells of the hemopoietic tissue increases. In the spleens of healthy mice case of prolonged administration of methylcobalamin, hyperplasia of the lymphoid elements, an incr number of DNA-synthesizing cells, and an increase in their mitotic index were noted. The stability periods of the mitotic cycle of spleen lymphocytes in the presence of an increase in the size of the p

TABLE 1. Stimulating Effects of Methylcobalamin on the Growth of Transplantable Tumors of Mice

Tumor	Line of mice	Dose of preparation, μg/kg	Increase in tumor volume after administration of methylcobalamin, % of control		
			7-8th day*	12-14th day*	21st day*
Ca-755	BDF_1	10	—	—	—10†
	C_3;BL	10	+75	+40†	+77
	F^A	500	+45	+15†	+50
	F_1	500	+77	+29†	+52
AKATOL	BALB/c	10	+126	+37	—33
RShM-5	CBA	10	+47	0	0
Sarcoma 37	SHK	500	+57	0	0

*Period after transplantation of tumor.
†P > 0.05, in all remaining cases P < 0.05.
Note. Here and in Table 2: the preparation was administered on the second and sixth days after transplantation of the tumor. A "plus" sign denotes stimulation of tumor growth.

Oncological Scientific Center of the Academy of Medical Sciences of the USSR. Scientific-Industrial Vitamin Combine, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 12, No. 10, pp. 49-54, October, 1978. Original article submitted April 3, 1978.

0091-150X/78/1210-1299 $07.50 © 1979 Plenum Publishing Corporation

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Exhibit 1023-0001

TABLE 2. Results of Combined Action of Methylcobalamin and Methotrexate on the Growth of Ca-755 (BDF_1)

Preparations	Dose of preparation	Inhibition of tumor growth* after course of administration of preparations, % of control		Increase in lifetime of animals, %
		1st-2nd day†	7-8th day†	
Methotrexate	10 mg/kg	94	51	19‡
Methylcobalamin	10 μg/kg	+180	+65	0
Methylcobalamin + methotrexate	10 mg/kg (simultaneously) 10 μg/kg	94	76	60
Methylcobalamin + methotrexate	10 μg/kg (methotrexate was administrated 6 h after methylcobalamin)	+36	+62	21‡

*Average results of five series of experiments.
†Period after transplantation of tumor.
‡P > 0.05; in all remaining cases P < 0.05. In the case of combined influence, the results obtained were evaluated relative to methotrexate.

Ex. 1023, Arsenyan, 2; Ex. 1075, Schiff Reply, ¶ 90

Prior Art: Sofyina (Ex. 1026)

POSSIBILITY OF POTENTIATING THE ANTINEOPLASTIC ACTION OF FOLIC ACID ANTAGONIST BY METHYLCOBALAMINE ANALOGUES

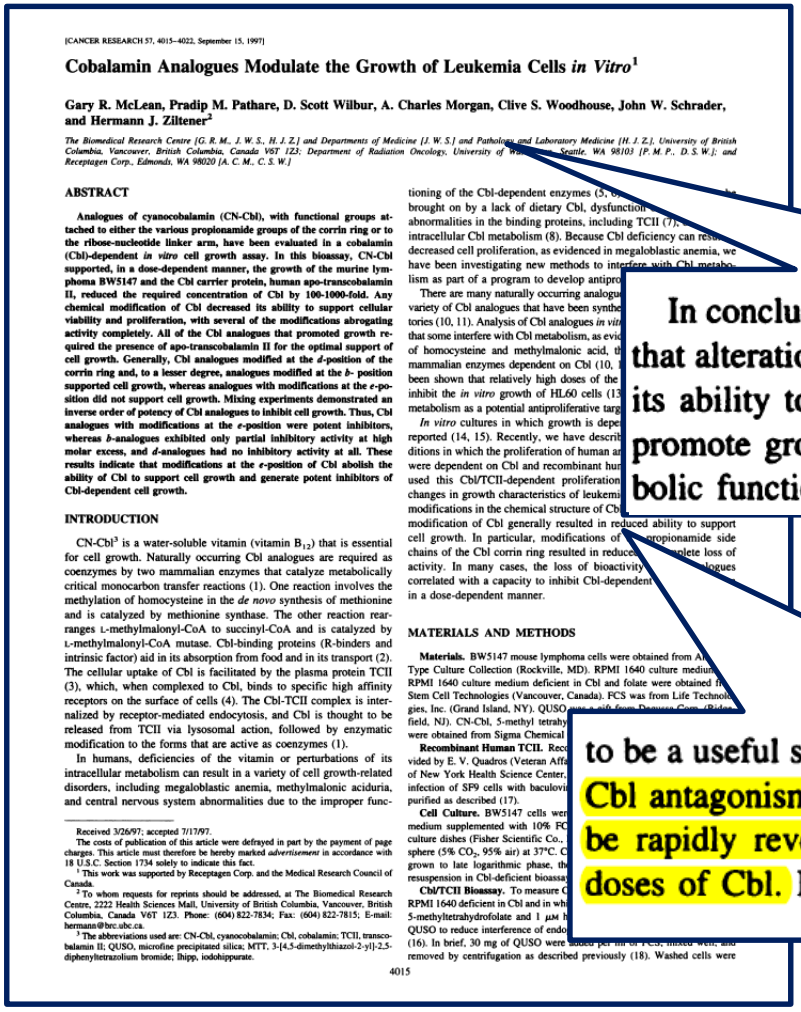
Z. P. Sofyina, N. V. Myasischeva, F. G. Arsenyan, A. M. Yurkevich

Summary. The effect of methylcobalamine and its analogues (difluoro-chloromethylcobalamine — CF_2ClCbl and methylcobalamine chloropalladate — $MetCbl \cdot DdCl_3$) on the growth of transplantable tumours in mice: adenocarcinoma of the mammary gland (Ca-755), carcinoma of the uterine cervix (CUC-5), carcinoma of the intestine (ACATOL) was studied. The activity of the cobalamine coenzyme analogues was investigated when used alone or combined with inhibitors of dehydrofolate reductase and methyonine synthetase. The results of the experiments indicate a stimulating effect of methylcobalamine on the growth of transplantable solid tumours in the animal organism. The antitumour activity of the methylcobalamine analogues studied was found to be higher in combined application with methotrexate. The most effective inhibition of tumour growth and the longer survival of the animals were achieved in combined application of methylcobalamine with methotrexate and methyonine synthetase inhibitor, depending upon the scheme of administration.

application with methotrexate. The most effective inhibition of tumour growth and the longer survival of the animals were achieved in combined application of methylcobalamine with methotrexate and methyonine synthetase inhibitor, depending upon the scheme of administration.

Ex. 1026, Sofyina, 7; Ex. 1075, Schiff Reply, ¶ 91

Prior Art: McLean (Ex. 2058)



[CANCER RESEARCH 57, 4015–4022, September 15, 1997]

Cobalamin Analogues Modulate the Growth of Leukemia Cells *in Vitro*[1]

Gary R. McLean, Pradip M. Pathare, D. Scott Wilbur, A. Charles Morgan, Clive S. Woodhouse, John W. Schrader, and Hermann J. Ziltener[2]

The Biomedical Research Centre [G. R. M., J. W. S., H. J. Z.] and Departments of Medicine [J. W. S.] and Pathology and Laboratory Medicine [H. J. Z.], University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3; Department of Radiation Oncology, University of Washington, Seattle, WA 98103 [P. M. P., D. S. W.]; and Receptagen Corp., Edmonds, WA 98020 [A. C. M., C. S. W.]

ABSTRACT

Analogues of cyanocobalamin (CN-Cbl), with functional groups attached to either the various propionamide groups of the corrin ring or to the ribose-nucleotide linker arm, have been evaluated in a cobalamin (Cbl)-dependent *in vitro* cell growth assay. In this bioassay, CN-Cbl supported, in a dose-dependent manner, the growth of the murine lymphoma BW5147 and the Cbl carrier protein, human apo-transcobalamin II, reduced the required concentration of Cbl by 100-1000-fold. Any chemical modification of Cbl decreased its ability to support cellular viability and proliferation, with several of the modifications abrogating activity completely. All of the Cbl analogues that promoted growth required the presence of apo-transcobalamin II for the optimal support of cell growth. Generally, Cbl analogues modified at the *d*-position of the corrin ring and, to a lesser degree, analogues modified at the *b*- position supported cell growth, whereas analogues with modifications at the *e*-position did not support cell growth. Mixing experiments demonstrated an inverse order of potency of Cbl analogues to inhibit cell growth. Thus, Cbl analogues with modifications at the *e*-position were potent inhibitors, whereas *b*-analogues exhibited only partial inhibitory activity at high molar excess, and *d*-analogues had no inhibitory activity at all. These results indicate that modifications at the *e*-position of Cbl abolish the ability of Cbl to support cell growth and generate potent inhibitors of Cbl-dependent cell growth.

INTRODUCTION

CN-Cbl[3] is a water-soluble vitamin (vitamin B_{12}) that is essential for cell growth. Naturally occurring Cbl analogues are required as coenzymes by two mammalian enzymes that catalyze metabolically critical monocarbon transfer reactions (1). One reaction involves the methylation of homocysteine in the *de novo* synthesis of methionine and is catalyzed by methionine synthase. The other reaction rearranges L-methylmalonyl-CoA to succinyl-CoA and is catalyzed by L-methylmalonyl-CoA mutase. Cbl-binding proteins (R-binders and intrinsic factor) aid in its absorption from food and in its transport (2). The cellular uptake of Cbl is facilitated by the plasma protein TCII (3), which, when complexed to Cbl, binds to specific high affinity receptors on the surface of cells (4). The Cbl-TCII complex is internalized by receptor-mediated endocytosis, and Cbl is thought to be released from TCII via lysosomal action, followed by enzymatic modification to the forms that are active as coenzymes (1).

In humans, deficiencies of the vitamin or perturbations of its intracellular metabolism can result in a variety of cell growth-related disorders, including megaloblastic anemia, methylmalonic aciduria, and central nervous system abnormalities due to the improper functioning of the Cbl-dependent enzymes (5, 6). [illegible] brought on by a lack of dietary Cbl, dysfunction [illegible] abnormalities in the binding proteins, including TCII (7), [illegible] intracellular Cbl metabolism (8). Because Cbl deficiency can result [illegible] decreased cell proliferation, as evidenced in megaloblastic anemia, we have been investigating new methods to interfere with Cbl metabolism as part of a program to develop antiproliferative [illegible]

There are many naturally occurring analogues [illegible] variety of Cbl analogues that have been synthesized [illegible] tories (10, 11). Analysis of Cbl analogues *in vitro* [illegible] that some interfere with Cbl metabolism, as evidenced [illegible] of homocysteine and methylmalonic acid, the [illegible] mammalian enzymes dependent on Cbl (10, [illegible] been shown that relatively high doses of the [illegible] inhibit the *in vitro* growth of HL60 cells (13) [illegible] metabolism as a potential antiproliferative target [illegible]

In vitro cultures in which growth is dependent [illegible] reported (14, 15). Recently, we have described [illegible] ditions in which the proliferation of human [illegible] were dependent on Cbl and recombinant human [illegible] used this Cbl/TCII-dependent proliferation [illegible] changes in growth characteristics of leukemia [illegible] modifications in the chemical structure of Cbl [illegible] modification of Cbl generally resulted in reduced ability to support cell growth. In particular, modifications of [illegible] propionamide side chains of the Cbl corrin ring resulted in reduced [illegible] plete loss of activity. In many cases, the loss of bioactivity [illegible] logues correlated with a capacity to inhibit Cbl-dependent [illegible] in a dose-dependent manner.

MATERIALS AND METHODS

Materials. BW5147 mouse lymphoma cells were obtained from [illegible] Type Culture Collection (Rockville, MD). RPMI 1640 culture medium [illegible] RPMI 1640 culture medium deficient in Cbl and folate were obtained from [illegible] Stem Cell Technologies (Vancouver, Canada). FCS was from Life Technologies, Inc. (Grand Island, NY). QUSO [illegible] field, NJ). CN-Cbl, 5-methyl tetrahydrofolate [illegible] were obtained from Sigma Chemical [illegible]

Recombinant Human TCII. [illegible] vided by E. V. Quadros (Veteran Affairs [illegible] of New York Health Science Center. [illegible] infection of SF9 cells with baculovirus [illegible] purified as described (17).

Cell Culture. BW5147 cells were [illegible] medium supplemented with 10% FCS [illegible] culture dishes (Fisher Scientific Co., [illegible] sphere (5% CO_2, 95% air) at 37°C. Cells [illegible] grown to late logarithmic phase, then [illegible] resuspension in Cbl-deficient bioassay [illegible]

Cbl/TCII Bioassay. To measure Cbl [illegible] RPMI 1640 deficient in Cbl and in which [illegible] 5-methyltetrahydrofolate and 1 μM [illegible] QUSO to reduce interference of endogenous [illegible] (16). In brief, 30 mg of QUSO were added per ml of FCS, mixed well, and removed by centrifugation as described previously (18). Washed cells were

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[1] This work was supported by Receptagen Corp. and the Medical Research Council of Canada.

[2] To whom requests for reprints should be addressed, at The Biomedical Research Centre, 2222 Health Sciences Mall, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3. Phone: (604) 822-7834; Fax: (604) 822-7815; E-mail: hermann@brc.ubc.ca.

[3] The abbreviations used are: CN-Cbl, cyanocobalamin; Cbl, cobalamin; TCII, transcobalamin II; QUSO, microfine precipitated silica; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Ihipp, iodohippurate.

In conclusion, we have demonstrated in an *in vitro* bioassay system that alterations of the *e*-propionamide side chain, which did not affect its ability to bind TCII, resulted in a complete loss of its ability to promote growth, indicating that this site is critical for proper metabolic function of Cbl. Modification of Cbl at other sites resulted in

to be a useful strategy in cancer therapy, and the potential problem of Cbl antagonism inducing neurotoxicity and other side effects should be rapidly reversed through the administration of pharmacological doses of Cbl. It has been shown that inactivation of Cbl by nitrous

Ex. 2058, McLean 4020, 4021; Ex. 1075, Schiff Reply ¶93

Prior Art: ViDAL (Ex. 2032)

ViDAL statement relied on by Lilly:

DC CONTRAINDICATIONS

- History of allergy to cobalamins (vitamin B_{12} and related substances)
- Malignant tumor: due to the action of vitamin B_{12} on the growth of tissues with a high rate of cellular multiplication, the risk of exacerbation must be taken into account

Ex. 2032, Vidal, 24

A. No. I, unfortunately, don't read French.

Q. Did -- have you reviewed the Physicians Desk Reference, though? Did you go looking for that?

A. I think I did. Yes.

Q. And you didn't find it, did you?

A. No, I didn't.

Ex. 1074, Chabner Dep., 106:5-107:4;
Ex. 1075, Schiff Reply, ¶ 94

A The PDR didn't state that B12 was contraindicated because of its effect on rapidly dividing cells.

Q Do you consider the PDR to be an important reference?

A It's one of many medical references that a POSA would be aware of.

Ex. 1086, Zeisel Dep., 42:4-25; Ex. 1075, Schiff Reply, ¶ 94

Prior Art: Non Prescription Physician's Desk Reference ("PDR") (Ex. 1106)

CANCER, NUTRIENTS DEFICIENCY SECONDARY TO

Cancer may be treated with chemotherapeutic agents. The following products may be recommended for relief of nutrients deficiency:

NOVARTIS/843

Amount Per Tablet	% Daily Value for Children 2–4 Years of Age	% Daily Value for Adults and Children 4 or More Years of Age
Vitamin A 5000 I.U.	100%	100%
Vitamin C 60 mg	80%	100%
Vitamin D 400 I.U.	50%	100%
Vitamin E 30 I.U.	150%	100%
Vitamin K 10 mcg	*	*
Thiamin 1.5 mg	110%	100%
Riboflavin 1.7 mg	110%	100%
Niacin 20 mg	110%	100%
Vitamin B_6 2 mg	140%	100%
Folate 0.4 mg	100%	100%
Vitamin B_{12}	100%	100%

Folate 0.4 mg
Vitamin B_{12} 6 mcg

Ex. 1106, Non-Prescription PDR, 403, 843; Ex. 1075, Schiff Reply, ¶ 95

Prior Art: PDR (Ex. 1092)

NASCOBAL™
[nās'cobal]
(Cyanocobalamin, USP)
Gel for Intranasal Administration

DESCRIPTION

Cyanocobalamin is a synthetic form of vitamin B_{12} with equivalent vitamin B_{12} activity. The chemical name is 5,6-dimethyl-benzimidazolyl cyanocobamide. The cobalt content is 4.35%. The molecular formula is $C_{63}H_{88}CoN_{14}O_{14}P$, which corresponds to a molecular weight of 1355.38 and the following structural formula:

initial priming, each metered gel delivers an average of 500 mcg of cyanocobalamin and the 5 mL bottle will deliver 8 doses of NASCOBAL™. If not used for 48 hours or longer,

CONTRAINDICATION

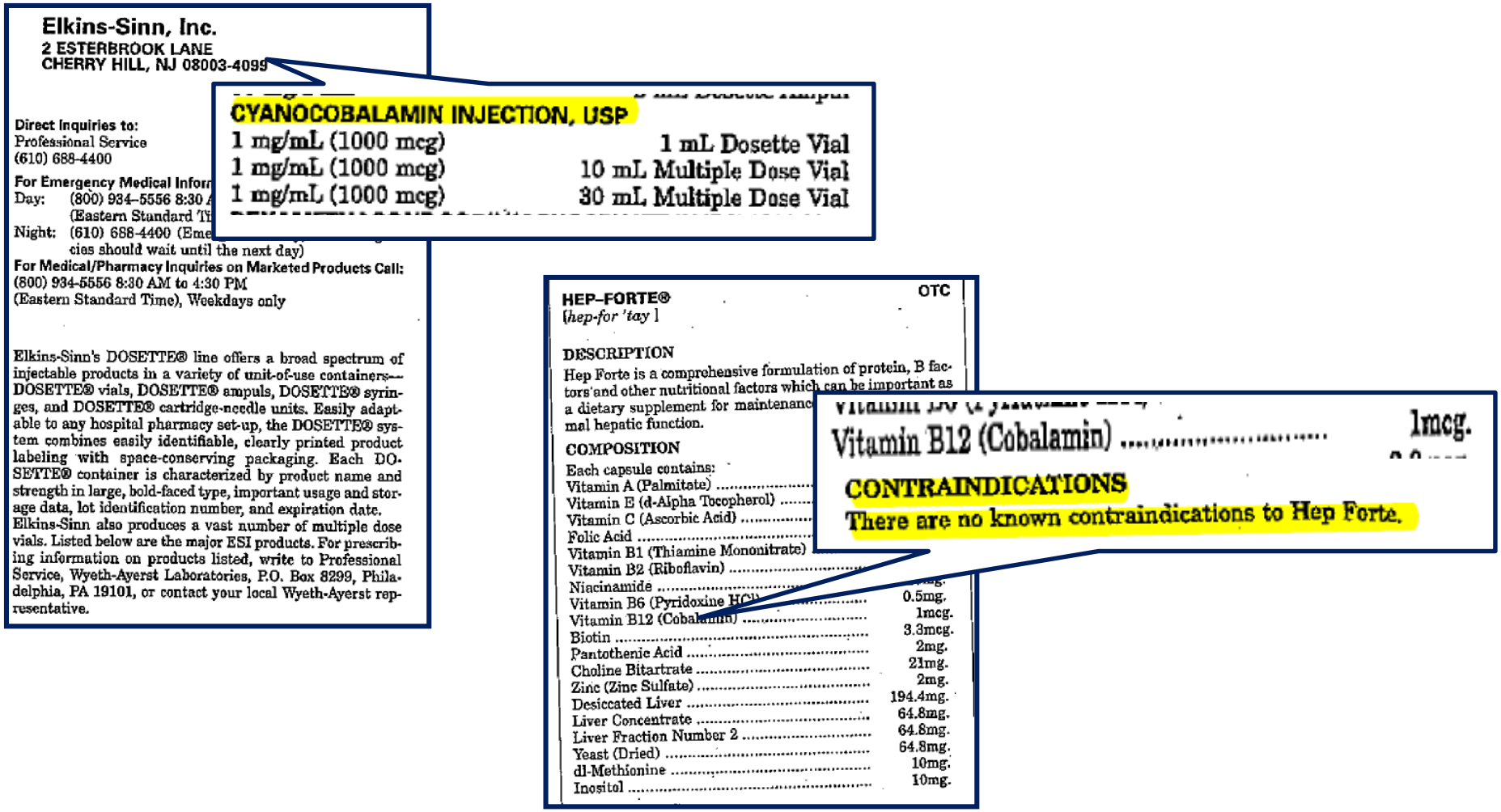
Sensitivity to cobalt and/or vitamin B_{12} or any component of the medication is a contraindication.

5. CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Long-term studies in animals to evaluate carcinogenic potential have not been done. There is no evidence from long-term use in patients with pernicious anemia that vitamin B_{12} is carcinogenic. Pernicious anemia is associated with an increased incidence of carcinoma of the stomach, but this is believed to be related to the underlying pathology and not to treatment with vitamin B_{12}.

Ex. 1092, 1999 PDR, 2914-2915; Ex. 1075, Schiff Reply, ¶ 95

Prior Art: PDR (Ex. 1092) (cont'd)



Elkins-Sinn, Inc.
2 ESTERBROOK LANE
CHERRY HILL, NJ 08003-4099

Direct Inquiries to:
Professional Service
(610) 688-4400

For Emergency Medical Inform
Day: (800) 934-5556 8:30 A
(Eastern Standard Ti
Night: (610) 688-4400 (Eme
cies should wait until the next day)
For Medical/Pharmacy Inquiries on Marketed Products Call:
(800) 934-5556 8:30 AM to 4:30 PM
(Eastern Standard Time), Weekdays only

Elkins-Sinn's DOSETTE® line offers a broad spectrum of injectable products in a variety of unit-of-use containers—DOSETTE® vials, DOSETTE® ampuls, DOSETTE® syringes, and DOSETTE® cartridge-needle units. Easily adaptable to any hospital pharmacy set-up, the DOSETTE® system combines easily identifiable, clearly printed product labeling with space-conserving packaging. Each DOSETTE® container is characterized by product name and strength in large, bold-faced type, important usage and storage data, lot identification number, and expiration date. Elkins-Sinn also produces a vast number of multiple dose vials. Listed below are the major ESI products. For prescribing information on products listed, write to Professional Service, Wyeth-Ayerst Laboratories, P.O. Box 8299, Philadelphia, PA 19101, or contact your local Wyeth-Ayerst representative.

CYANOCOBALAMIN INJECTION, USP

1 mg/mL (1000 mcg)	1 mL Dosette Vial
1 mg/mL (1000 mcg)	10 mL Multiple Dose Vial
1 mg/mL (1000 mcg)	30 mL Multiple Dose Vial

HEP–FORTE® OTC
(*hep-for 'tay*)

DESCRIPTION
Hep Forte is a comprehensive formulation of protein, B factors and other nutritional factors which can be important as a dietary supplement for maintenanc
mal hepatic function.

COMPOSITION
Each capsule contains:

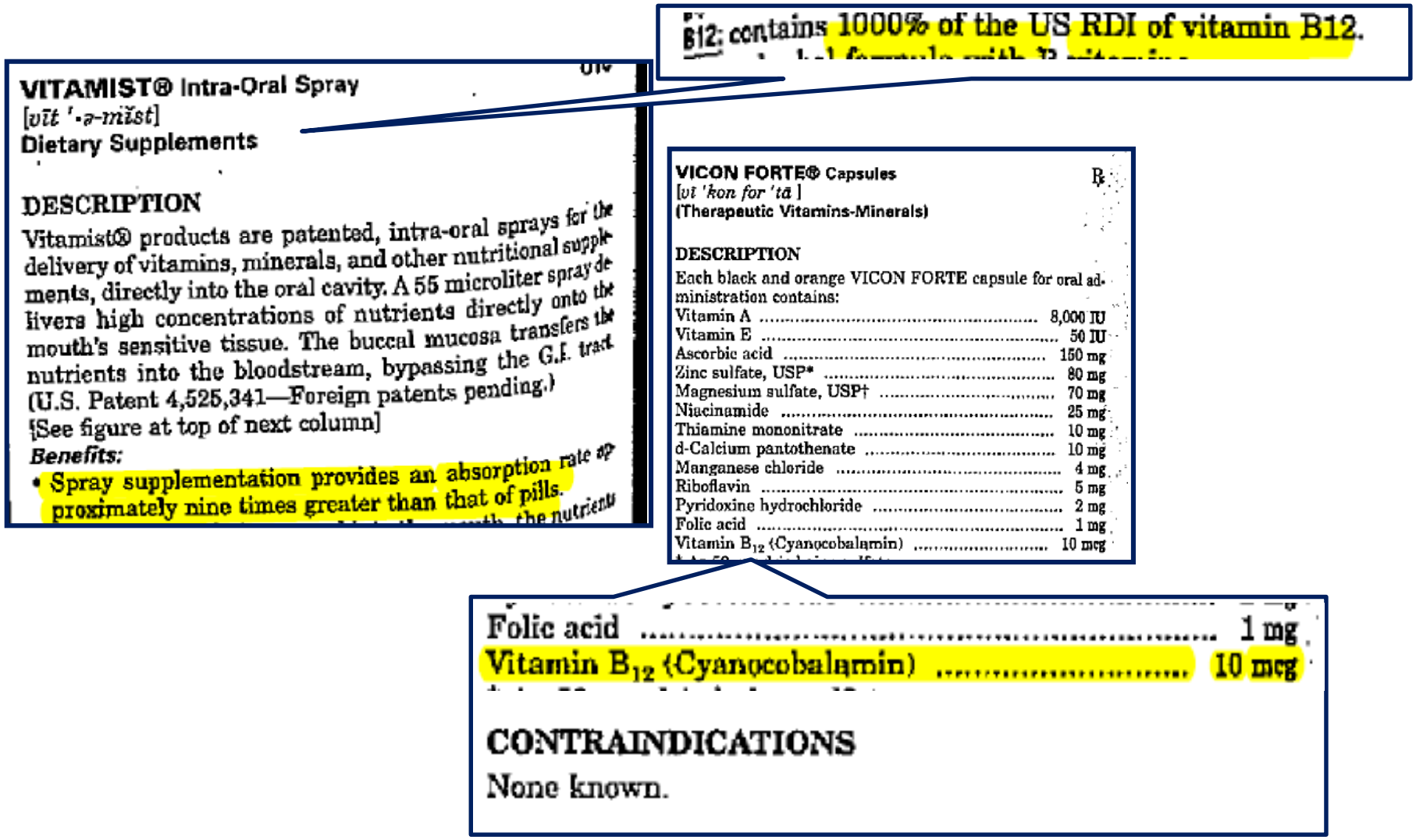
Vitamin A (Palmitate)	
Vitamin E (d-Alpha Tocopherol)	
Vitamin C (Ascorbic Acid)	
Folic Acid	
Vitamin B1 (Thiamine Mononitrate)	
Vitamin B2 (Riboflavin)	
Niacinamide	
Vitamin B6 (Pyridoxine HCl)	0.5mg.
Vitamin B12 (Cobalamin)	1mcg.
Biotin	3.3mcg.
Pantothenic Acid	2mg.
Choline Bitartrate	21mg.
Zinc (Zinc Sulfate)	2mg.
Desiccated Liver	194.4mg.
Liver Concentrate	64.8mg.
Liver Fraction Number 2	64.8mg.
Yeast (Dried)	64.8mg.
dl-Methionine	10mg.
Inositol	10mg.

Vitamin B12 (Cobalamin) ..... 1mcg.

CONTRAINDICATIONS
There are no known contraindications to Hep Forte.

Ex. 1092, 1999 PDR, 403, 843; Ex. 1075, Schiff Reply, ¶ 95

Prior Art: PDR (Ex. 1092)



B12: contains 1000% of the US RDI of vitamin B12.

VITAMIST® Intra-Oral Spray
[vīt '-ə-mĭst]
Dietary Supplements

DESCRIPTION

Vitamist® products are patented, intra-oral sprays for the delivery of vitamins, minerals, and other nutritional supplements, directly into the oral cavity. A 55 microliter spray delivers high concentrations of nutrients directly onto the mouth's sensitive tissue. The buccal mucosa transfers the nutrients into the bloodstream, bypassing the G.I. tract. (U.S. Patent 4,525,341—Foreign patents pending.)
[See figure at top of next column]

Benefits:

- Spray supplementation provides an absorption rate approximately nine times greater than that of pills.

VICON FORTE® Capsules ℞
[vī 'kon for 'tā]
(Therapeutic Vitamins-Minerals)

DESCRIPTION

Each black and orange VICON FORTE capsule for oral administration contains:

Vitamin A	8,000 IU
Vitamin E	50 IU
Ascorbic acid	150 mg
Zinc sulfate, USP*	80 mg
Magnesium sulfate, USP†	70 mg
Niacinamide	25 mg
Thiamine mononitrate	10 mg
d-Calcium pantothenate	10 mg
Manganese chloride	4 mg
Riboflavin	5 mg
Pyridoxine hydrochloride	2 mg
Folic acid	1 mg
Vitamin B_{12} (Cyanocobalamin)	10 mcg

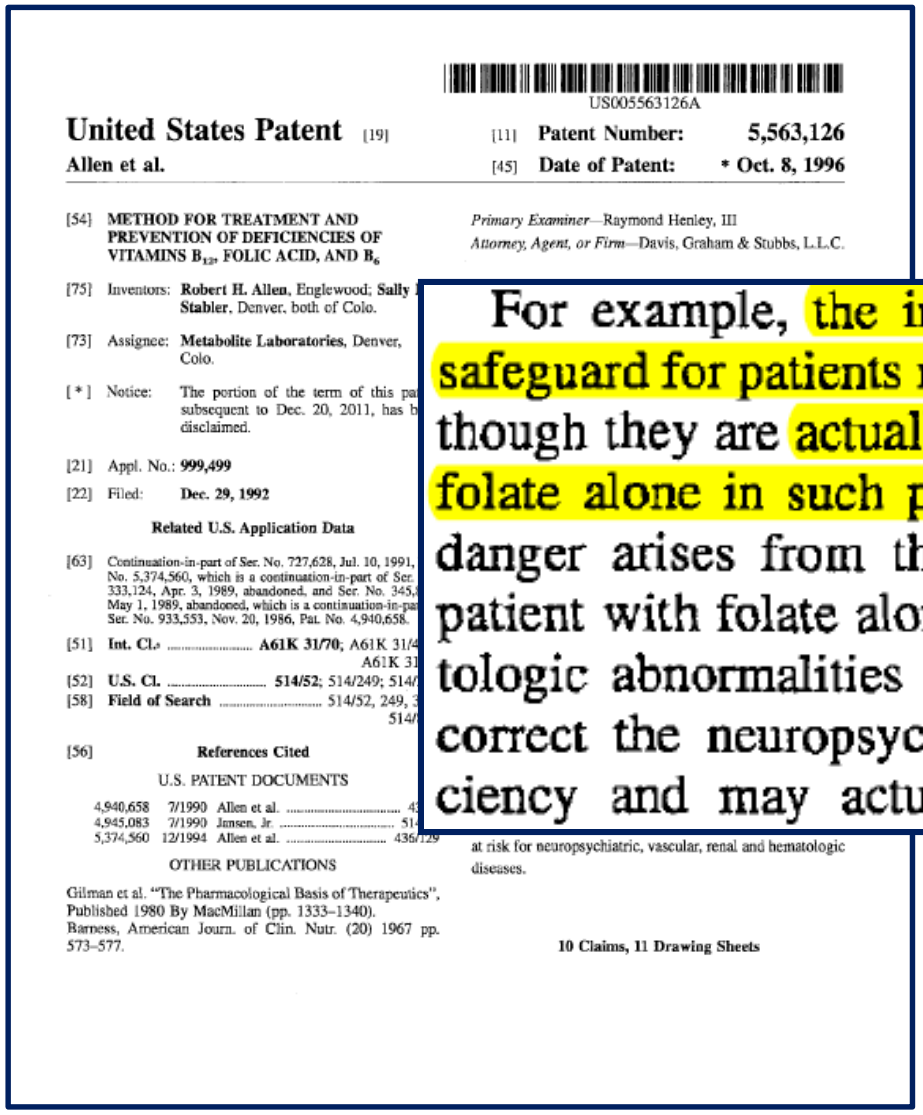
Folic acid ..... 1 mg
Vitamin B_{12} (Cyanocobalamin) ..... 10 mcg

CONTRAINDICATIONS

None known.

Ex. 1092, 1999 PDR, 1666, 3170; Ex. 1075, Schiff Reply, ¶ 95

Prior Art: Allen (Ex. 1018) and Masking: "[T]reatment with folate alone in such patients is extremely dangerous"



US005563126A

United States Patent [19]

Allen et al.

[11] **Patent Number: 5,563,126**

[45] **Date of Patent: * Oct. 8, 1996**

[54] **METHOD FOR TREATMENT AND PREVENTION OF DEFICIENCIES OF VITAMINS B_{12}, FOLIC ACID, AND B_6**

Primary Examiner—Raymond Henley, III
Attorney, Agent, or Firm—Davis, Graham & Stubbs, L.L.C.

[75] Inventors: **Robert H. Allen**, Englewood; **Sally ... Stabler**, Denver, both of Colo.

[73] Assignee: **Metabolite Laboratories**, Denver, Colo.

[*] Notice: The portion of the term of this pa... subsequent to Dec. 20, 2011, has b... disclaimed.

[21] Appl. No.: **999,499**

[22] Filed: **Dec. 29, 1992**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 727,628, Jul. 10, 1991, ... No. 5,374,560, which is a continuation-in-part of Ser. ... 333,124, Apr. 3, 1989, abandoned, and Ser. No. 345,... May 1, 1989, abandoned, which is a continuation-in-pa... Ser. No. 933,553, Nov. 20, 1986, Pat. No. 4,940,658.

[51] **Int. Cl.** ..... **A61K 31/70**; A61K 31/4... A61K 31...

[52] **U.S. Cl.** ..... **514/52**; 514/249; 514/...

[58] **Field of Search** ..... 514/52, 249, 3... 514/...

[56] **References Cited**

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Barness, American Journ. of Clin. Nutr. (20) 1967 pp. 573–577.

at risk for neuropsychiatric, vascular, renal and hematologic diseases.

10 Claims, 11 Drawing Sheets

For example, the inclusion of B_{12} will be useful as a safeguard for patients misdiagnosed as folate deficient, even though they are actually B_{12} deficient, since treatment with folate alone in such patients is extremely dangerous. The danger arises from the fact that treating a B_{12} deficient patient with folate alone may reverse or prevent the hematologic abnormalities seen in B_{12} deficiency, but will not correct the neuropsychiatric abnormalities of a B_{12} deficiency and may actually precipitate them. Even in the

Ex. 1018, '126 Patent 7:51-59; Ex. 1004, Schiff Decl., ¶ 83

Prior Art Disclosures

STANDARD DOSES OF FOLIC ACID + VITAMIN B_{12}

'209 Patent: Narrowest Claimed Dosage Ranges

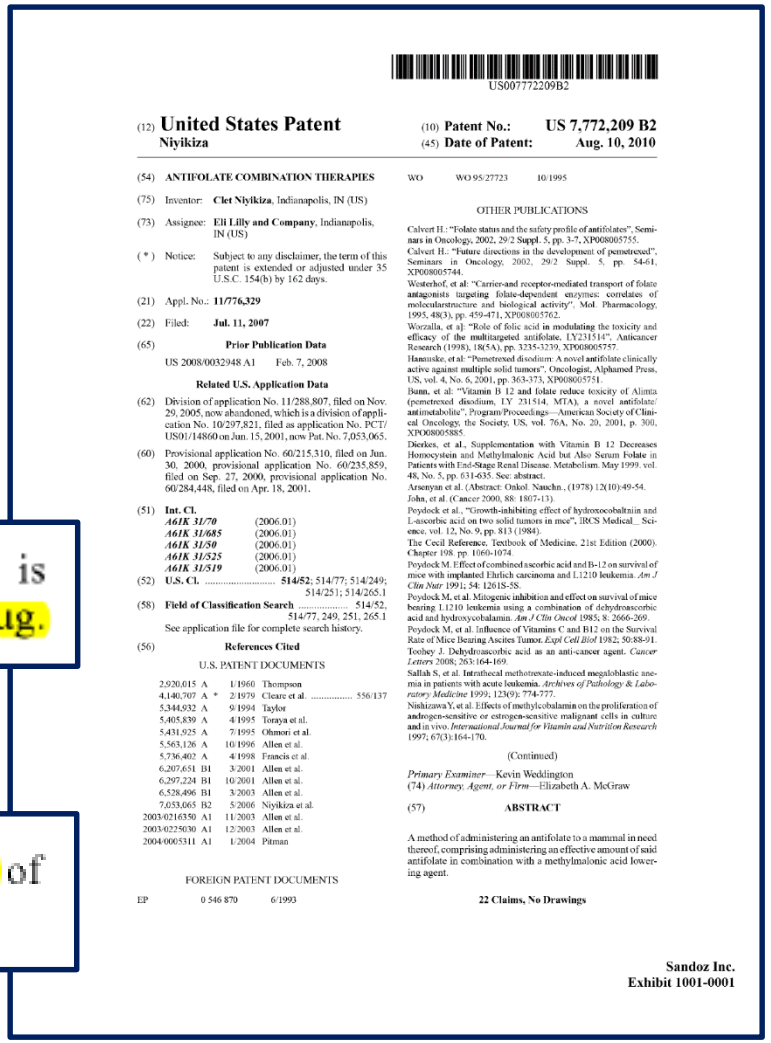
- Dependent claims 15 and 18 exemplary of narrowest dosages

Claim 15: narrowest B_{12} dosage

15. The method of claim **14**, wherein vitamin B12 is administered as an intramuscular injection of about 1000 µg.

Claim 18: narrowest folic acid dosage

18. The method of claim **17** wherein 350 µg to 600 µg of folic acid is administered.



US007772209B2

(12) **United States Patent**
Niyikiza

(10) **Patent No.: US 7,772,209 B2**
(45) **Date of Patent: Aug. 10, 2010**

(54) **ANTIFOLATE COMBINATION THERAPIES**

(75) Inventor: **Clet Niyikiza**, Indianapolis, IN (US)

(73) Assignee: **Eli Lilly and Company**, Indianapolis, IN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 162 days.

(21) Appl. No.: **11/776,329**

(22) Filed: **Jul. 11, 2007**

(65) **Prior Publication Data**

US 2008/0032948 A1 Feb. 7, 2008

Related U.S. Application Data

(62) Division of application No. 11/288,807, filed on Nov. 29, 2005, now abandoned, which is a division of application No. 10/297,821, filed as application No. PCT/US01/14860 on Jun. 15, 2001, now Pat. No. 7,053,065.

(60) Provisional application No. 60/215,310, filed on Jun. 30, 2000, provisional application No. 60/235,859, filed on Sep. 27, 2000, provisional application No. 60/284,448, filed on Apr. 18, 2001.

(51) **Int. Cl.**
A61K 31/70 (2006.01)
A61K 31/685 (2006.01)
A61K 31/50 (2006.01)
A61K 31/525 (2006.01)
A61K 31/519 (2006.01)

(52) **U.S. Cl.** **514/52**; 514/77; 514/249; 514/251; 514/265.1

(58) **Field of Classification Search** 514/52, 514/77, 249, 251, 265.1
See application file for complete search history.

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(Continued)

Primary Examiner—Kevin Weddington
(74) *Attorney, Agent, or Firm*—Elizabeth A. McGraw

(57) **ABSTRACT**

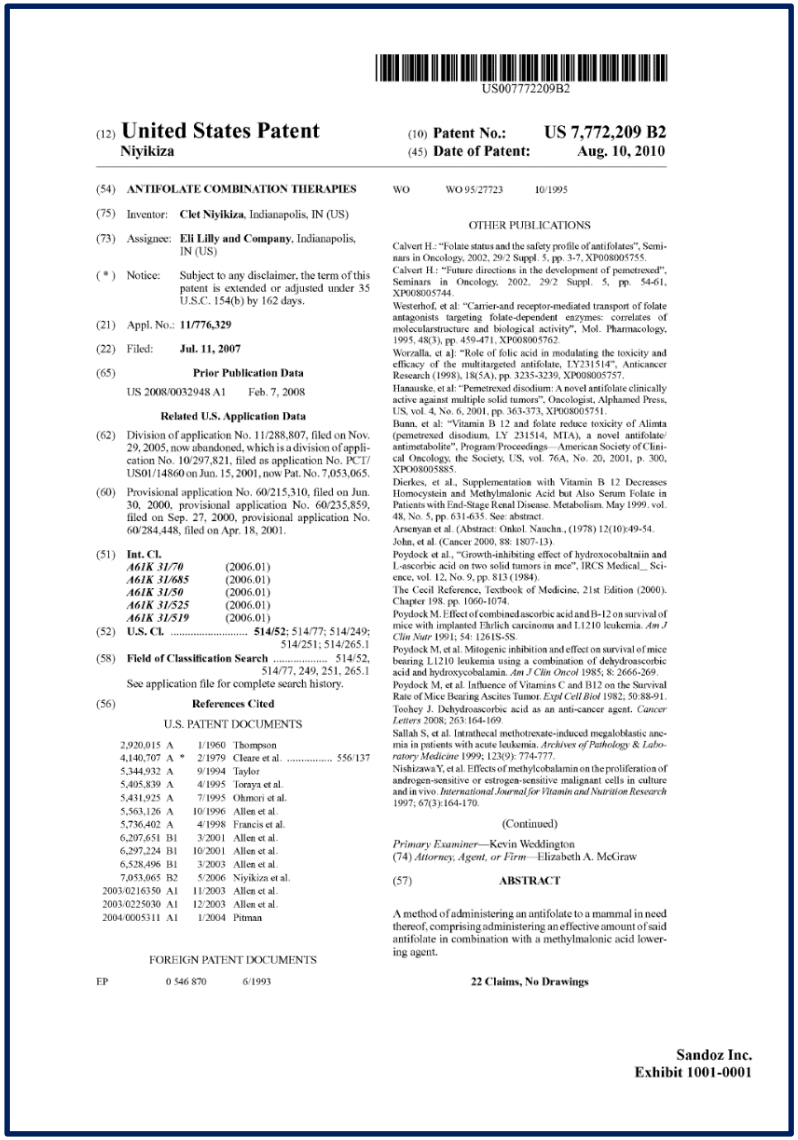
A method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1001-0001

Ex. 1001

‘209 Patent



US007772209B2

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Niyikiza

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Sandoz Inc.
Exhibit 1001-0001

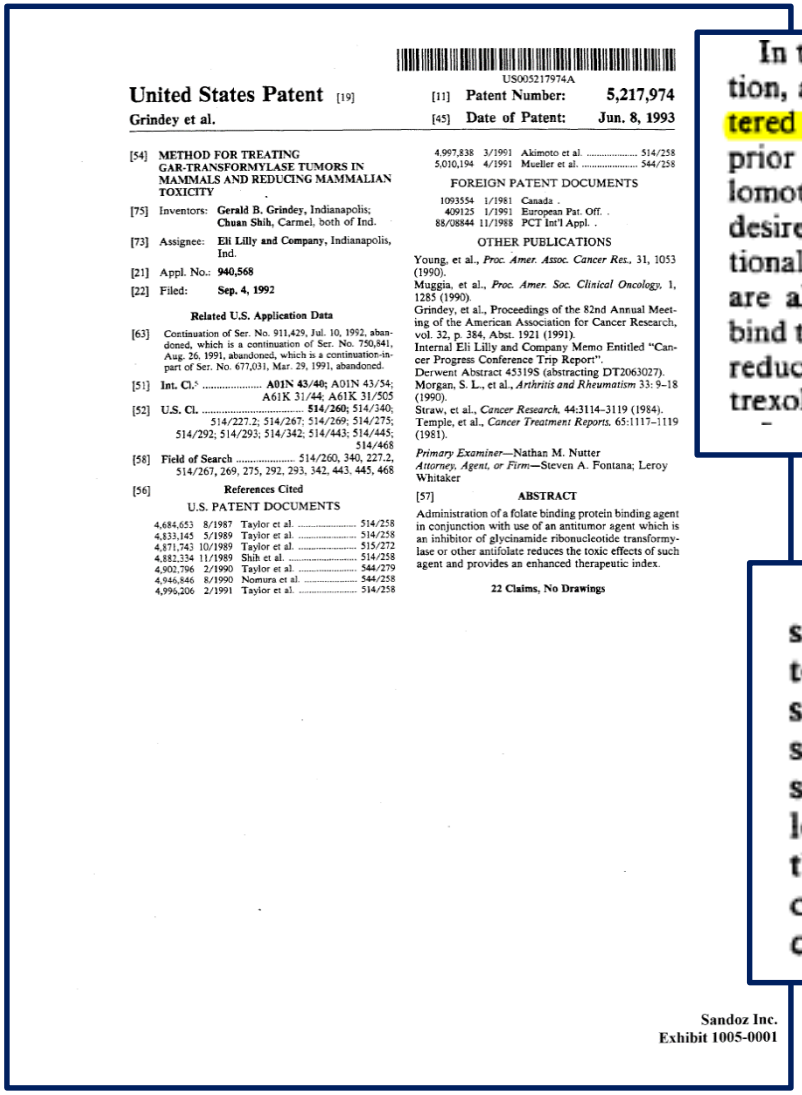
In the especially preferred embodiment of this invention, about 0.1 mg to about 30 mg, most preferably about 0.3 mg to about 5 mg, of folic acid is administered orally to a mammal about 1 to 3 weeks post administration of the methylmalonic acid lowering agent and about 1 to about 24 hours prior to the parenteral administration of the amount of an antifolate. However, it will be understood that the amount of the methylmalonic acid lowering agent actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual agent administered, the age, weight, and response of the individual patient, and the severity of the patient’s symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect.

Ex. 1001, ‘209 patent, col. 6, ll. 35-53; Ex. 1004, Schiff Decl. ¶¶ 19-20.

Prior Art Disclosures

STANDARD DOSES OF FOLIC ACID

Folic Acid Dosage: '974 patent and dosages within 350-600 µg



US005217974A

United States Patent [19]
Grindey et al.

[11] **Patent Number: 5,217,974**
[45] **Date of Patent: Jun. 8, 1993**

[54] **METHOD FOR TREATING GAR-TRANSFORMYLASE TUMORS IN MAMMALS AND REDUCING MAMMALIAN TOXICITY**

[75] Inventors: **Gerald B. Grindey**, Indianapolis; **Chuan Shih**, Carmel, both of Ind.

[73] Assignee: **Eli Lilly and Company**, Indianapolis, Ind.

[21] Appl. No.: **940,568**

[22] Filed: **Sep. 4, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 911,429, Jul. 10, 1992, abandoned, which is a continuation of Ser. No. 750,841, Aug. 26, 1991, abandoned, which is a continuation-in-part of Ser. No. 677,031, Mar. 29, 1991, abandoned.

[51] **Int. Cl.**[5] **A01N 43/40**; A01N 43/54; A61K 31/44; A61K 31/505

[52] **U.S. Cl.** **514/260**; 514/340; 514/227.2; 514/267; 514/269; 514/275; 514/292; 514/293; 514/342; 514/443; 514/445; 514/468

[58] **Field of Search** 514/260, 340, 227.2, 514/267, 269, 275, 292, 293, 342, 443, 445, 468

[56] **References Cited**

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Derwent Abstract 45319S (abstracting DT2063027).
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Primary Examiner—Nathan M. Nutter
Attorney, Agent, or Firm—Steven A. Fontana; Leroy Whitaker

[57] **ABSTRACT**

Administration of a folate binding protein binding agent in conjunction with use of an antitumor agent which is an inhibitor of glycinamide ribonucleotide transformylase or other antifolate reduces the toxic effects of such agent and provides an enhanced therapeutic index.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1005-0001

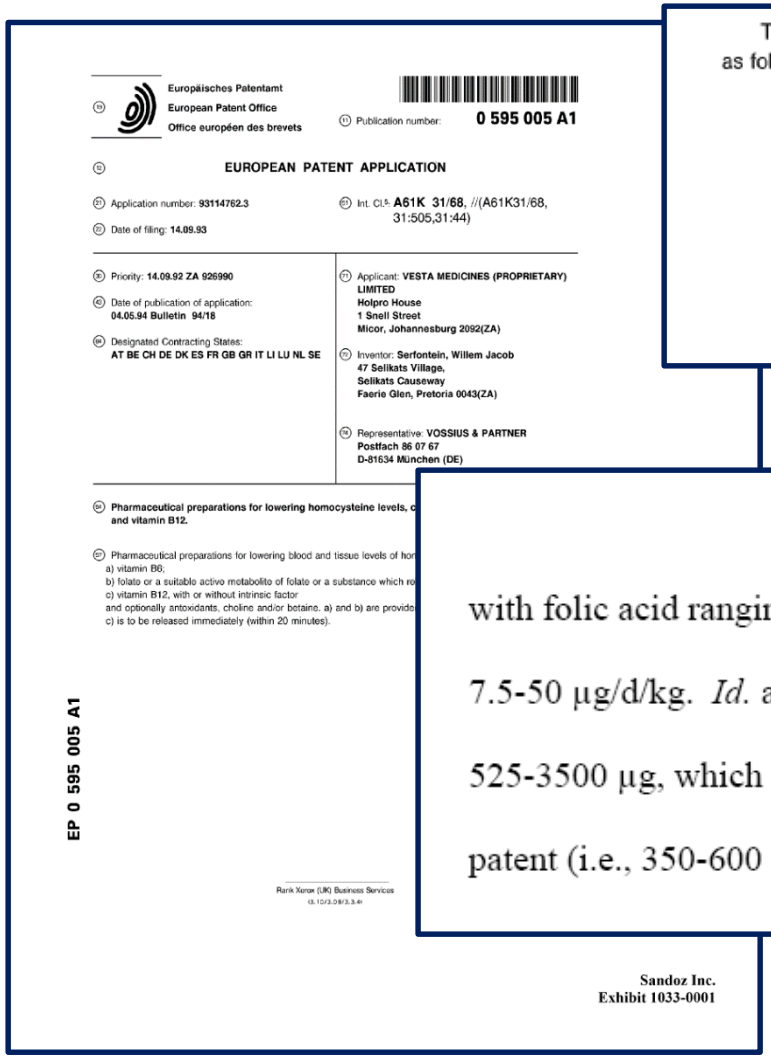
In the especially preferred embodiment of this invention, about 1 mg to about 5 mg of folic acid is administered orally to a mammal about 1 to about 24 hours prior to the parenteral administration of the amount of lomotrexol which is normally required to attain the desired therapeutic benefit. Although greater or additional doses of folic acid or another FBP binding agent are also operable, the above parameters will usually bind the folate binding protein in an amount sufficient to reduce the toxicity effects normally seen upon lomotrexol administration above.

Ex. 1005, col. 6, ll. 38-39; Schiff ¶ 93; Ex. 1075, Schiff Reply, ¶ 111.

In preparation for the foregoing clinical study, pilot studies in humans have established that folic acid given to patients receiving lometrexol has effected reduced side effects due to the lometrexol. Specifically, in one subject who had a nasalpharyngeal carcinoma, who was supplimented with folic acid at 0.5 to 1.0 mg/day, lometrexol was well tolerated for up to 12 months of therapy. Moreover, this patient has no clinical evidence of disease after the 12 months of therapy. These data are consistent with the animal studies reported above.

Ex. 1005, col. 8, ll. 49-59; Schiff ¶ 93; Ex. 1075, Schiff Reply, ¶ 111.

Folic Acid Dosage: European Patent Application 0 595 005 (EP005) (Ex. 1033) and dosages within 350-600 µg



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 595 005 A1**

EUROPEAN PATENT APPLICATION

(21) Application number: 93114762.3
(22) Date of filing: 14.09.93
(51) Int. Cl.5: A61K 31/68, //(A61K31/68, 31:505,31:44)

(30) Priority: 14.09.92 ZA 926990
(43) Date of publication of application: 04.05.94 Bulletin 94/18
(84) Designated Contracting States: AT BE CH DE DK ES FR GB GR IT LI LU NL SE

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(54) Pharmaceutical preparations for lowering homocysteine levels, c
and vitamin B12.

(57) Pharmaceutical preparations for lowering blood and tissue levels of hor
a) vitamin B6;
b) folate or a suitable active metabolite of folate or a substance which re
c) vitamin B12, with or without intrinsic factor
and optionally antioxidants, choline and/or betaine. a) and b) are provide
c) is to be released immediately (within 20 minutes).

EP 0 595 005 A1

Rank Xerox (UK) Business Services

Sandoz Inc.
Exhibit 1033-0001

The preparations in accordance with the invention are formulated to provide approximate daily dosages as follows (µg/d/kg body weight).

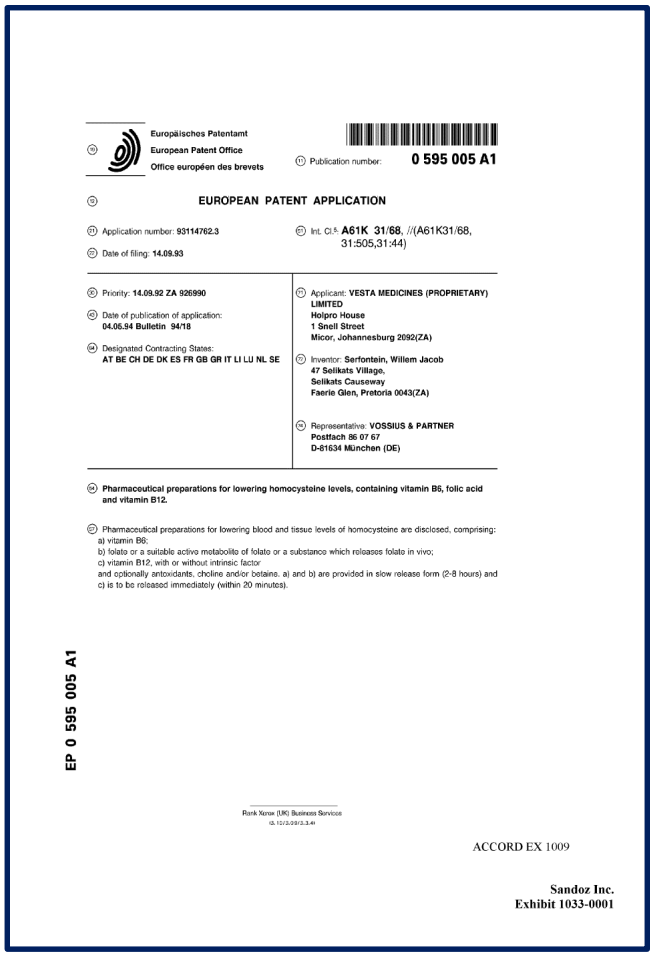
	a) Vitamin B6	b) Folic Acid	c) Vitamin B12
Broadest range	15-750	1,5-150	1,5-75
preferred range	30-400	7,5-50	3-15
more preferred range	75-250	10-30	7-10
typical example	150	15	7,5

Ex. 1033, EP 005 at 4-5, 9; Ex. 1004, Schiff Decl. ¶96

EP suggests treating high homocysteine with folic acid ranging from 1.5-150 µg/d/kg, with a more "preferred range" of 7.5-50 µg/d/kg. *Id.* at 5. For a 70 kg person, this preferred range corresponds to 525-3500 µg, which overlaps with the narrowest of the claimed ranges of the '209 patent (i.e., 350-600 µg of claims 10 and 18). Ex. 1033, EP005 at 3.

Ex. 1004, Schiff Decl. ¶96

Folic Acid Dosage: European Patent Application 0 595 005 (EP005) (Ex. 1033) and dosages within 350-600 µg



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(11) Publication number: **0 595 005 A1**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: **93114762.3**

(22) Date of filing: **14.09.93**

(51) Int. Cl.5: **A61K 31/68**, //(A61K31/68, 31:505,31:44)

(30) Priority: **14.09.92 ZA 926990**

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(84) Designated Contracting States: **AT BE CH DE DK ES FR GB GR IT LI LU NL SE**

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(74) Representative: **VOSSIUS & PARTNER**
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(54) **Pharmaceutical preparations for lowering homocysteine levels, containing vitamin B6, folic acid and vitamin B12.**

(57) Pharmaceutical preparations for lowering blood and tissue levels of homocysteine are disclosed, comprising:
a) vitamin B6;
b) folate or a suitable active metabolite of folate or a substance which releases folate in vivo;
c) vitamin B12, with or without intrinsic factor
and optionally antioxidants, choline and/or betaine. a) and b) are provided in slow release form (2-8 hours) and c) is to be released immediately (within 20 minutes).

EP 0 595 005 A1

Rank Xerox (UK) Business Services

ACCORD EX 1009

Sandoz Inc.
Exhibit 1033-0001

Ex. 1033 EP005 at 9; Paper 14, Institution at 15; Ex. 1004, Schiff Decl. ¶79; Ex. 1075, Schiff Reply, ¶¶78-82.

Concentration ranges of pyridoxal, folate and vitamin B12 in pharmaceutical formulations

The following quantities refer to one daily dose for an adult patient of approximately 70kg body weight. (PL=pyridoxal; Fol=folate; B12=Vitamin B12) Quantities are given in milligrams per day.

	PL		Folate		B12	
Formulation type	Range mg	Preferred mg	Range mg	Preferred mg	Range mg	Preferred mg
Normal (no absorption problem)	2-5	5	0,2-15	1,0	0.1-2	0.5
Special (to overcome absorption problems)	2-50	5	2-15	5	0.2-5	1,0

Folic Acid Dosage: Morgan (Ex. 1010) and dosages within 350-600 µg

Supplementation with Folic Acid during Methotrexate Therapy for Rheumatoid Arthritis

A Double-Blind, Placebo-controlled Trial

Sarah L. Morgan, MD, RD; Joseph E. Baggott, PhD; William H. Vaughn, BS; Janet S. Austin, MA; Tonya A. Veitch, BS; Jeannette Y. Lee, PhD; William J. Koopman, MD; Carlos L. Krumdieck, MD, PhD; and Graciela S. Alarcón, MD, MPH

■ *Objective:* To determine the effect of two different weekly doses of folic acid on the toxicity and efficacy of low-dose methotrexate therapy for rheumatoid arthritis.
■ *Design:* Randomized, double-blind, placebo-controlled study.
■ *Patients:* 79 persons between 19 and 78 years of age who fulfilled the American Rheumatism Association's criteria for rheumatoid arthritis.
■ *Intervention:* Participants were randomly assigned to visually identical placebo or to 5 mg or 27.5 mg of folic acid each week.
■ *Measurements:* Duration, intensity, and clinical severity of toxic events; efficacy (indices of joint tenderness and swelling and grip strength); plasma and erythrocyte folate levels; and other laboratory variables.
■ *Results:* Folic acid supplementation at either dose did not affect the efficacy of methotrexate therapy as judged by joint indices and patient and physician assessments of disease. Patients given folic acid supplements had lower toxicity scores than did participants given placebo ($P \leq 0.001$). Low blood folate levels and increased mean corpuscular volumes were associated with substantial methotrexate toxicity, whereas daily dietary intakes of more than 900 nmol (400 µg) of folic acid were associated with little methotrexate toxicity.
■ *Conclusions:* Folic acid, an inexpensive vitamin, is safe in a broad range of doses and protects patients with rheumatoid arthritis who are taking methotrexate from toxicity while preserving the efficacy of methotrexate.

Ann Intern Med. 1994;121:833-841.

From the Birmingham Veteran's Administration Hospital and the University of Alabama at Birmingham, Birmingham, Alabama. For current author addresses, see end of text.

The folic acid antagonist methotrexate (N-10-methylopterin) is useful in low doses (2.5 to 20 mg/wk treating chronic inflammatory diseases (1–7). Many have established the efficacy of methotrexate in rheumatoid arthritis (7–13). Compared with other disease-modifying drugs, methotrexate has the highest probability drug continuation at 10 years. Dose response–related toxic effects have been reported in 30% to 90% of patients given methotrexate (13). Toxic effects include gastrointestinal intolerance, hematologic abnormalities, alopecia, hepatotoxicity, and pulmonary toxicity (14–22).

Some side effects of methotrexate administration, such as gastrointestinal intolerance, mimic complicated folate deficiency (23). Folate deficiency occurs frequently in patients with rheumatoid arthritis; further, folate stores are decreased in patients with rheumatoid arthritis who take methotrexate, suggesting that impaired folate status is related to toxicity (24–26).

Folic acid supplementation has been reported anecdotally to lessen toxicity in patients receiving methotrexate treatment (27, 28). In a 6-month, double-blind, placebo-controlled trial, 7 mg of folic acid weekly (1 mg/d or 2265 nmol/d) decreased methotrexate toxicity without affecting efficacy (29). This was confirmed by Stewart and colleagues (30) in a retrospective chart review.

Folinic acid (leucovorin, citrovorum factor) is a one-carbon–substituted, fully reduced folate that has also been administered during methotrexate therapy (31–36). Low doses of the vitamin (1 to 7 mg/wk) have decreased methotrexate toxicity (35, 36). Higher doses negate efficacy and lessen toxicity (31, 32). Thus, the folinic acid dose may critically affect the efficacy of methotrexate therapy.

The influence of the folic acid dose on methotrexate toxicity and efficacy remains controversial, and the effects of different doses of folic acid are not known (37, 38). Some investigators argue that if toxic effects occur, the most rational approach is to reduce the dose of methotrexate rather than to provide folic acid supplements (37). We designed a larger and longer study to evaluate different doses of folic acid, assuming that toxicity could be reduced without changing the efficacy of methotrexate.

Methods

Participants

Patients aged 19 to 78 years who fulfilled the American College of Rheumatology's revised criteria for rheumatoid arthritis

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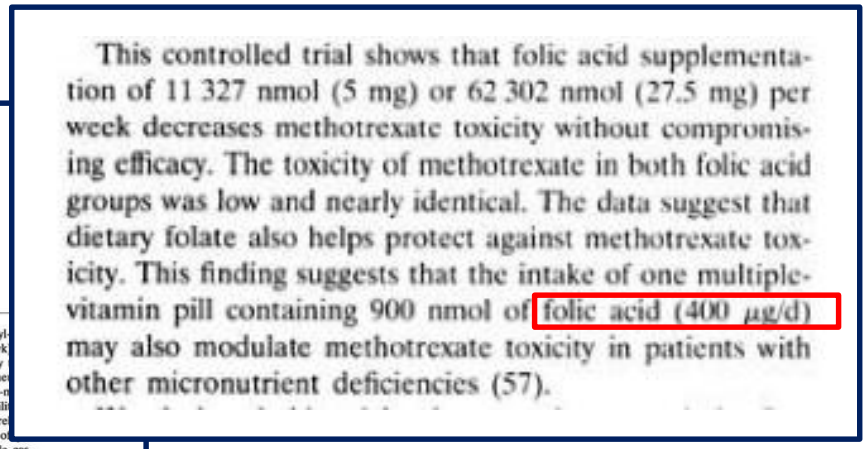
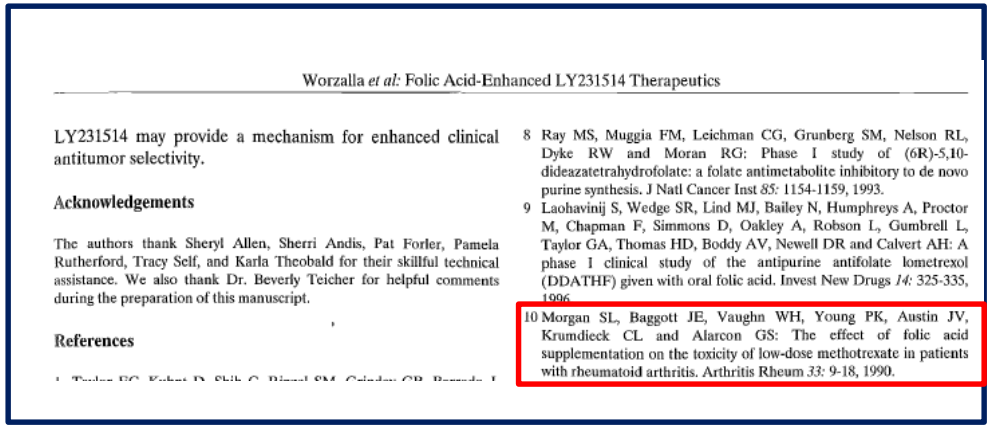
Sandoz Inc.
Exhibit 1010-0001



This controlled trial shows that folic acid supplementation of 11 327 nmol (5 mg) or 62 302 nmol (27.5 mg) per week decreases methotrexate toxicity without compromising efficacy. The toxicity of methotrexate in both folic acid groups was low and nearly identical. The data suggest that dietary folate also helps protect against methotrexate toxicity. This finding suggests that the intake of one multiple-vitamin pill containing 900 nmol of folic acid (400 µg/d) may also modulate methotrexate toxicity in patients with other micronutrient deficiencies (57).

Ex. 1010, Morgan at 838; Ex. 1004, Schiff Decl., ¶ 95; Ex. 1075, Schiff Reply, ¶ 109.



Worzalla *et al:* Folic Acid-Enhanced LY231514 Therapeutics

LY231514 may provide a mechanism for enhanced clinical antitumor selectivity.

Acknowledgements

The authors thank Sheryl Allen, Sherri Andis, Pat Forler, Pamela Rutherford, Tracy Self, and Karla Theobald for their skillful technical assistance. We also thank Dr. Beverly Teicher for helpful comments during the preparation of this manuscript.

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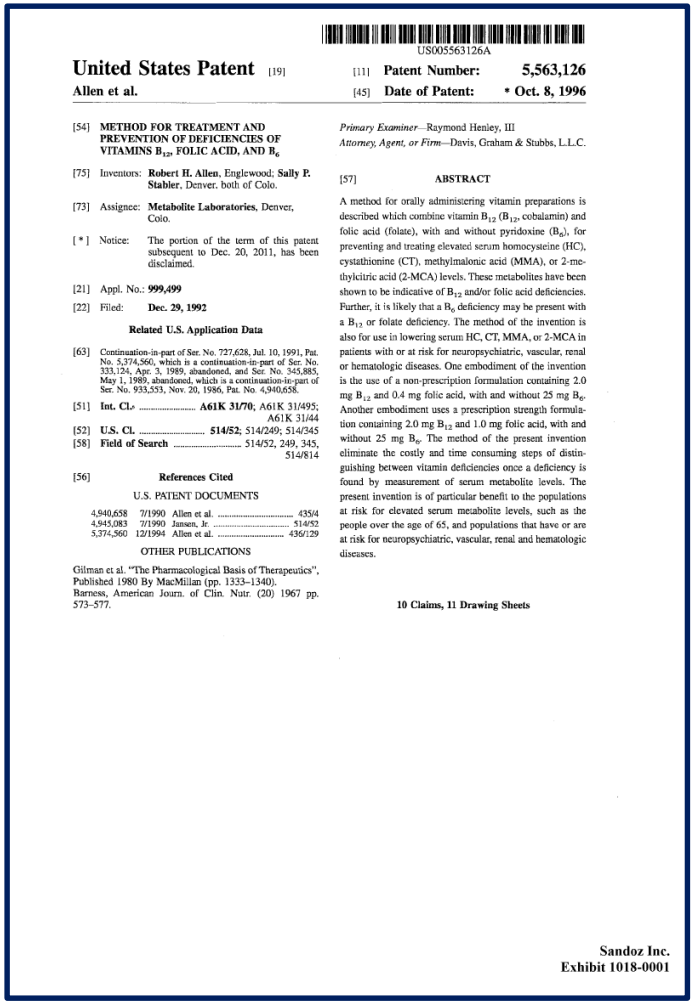
Ex. 1013, Worzalla at 3239 n.10 (citing Ex. 2084, Morgan); Ex. 1075, Schiff Reply, ¶ 109.

Folic Acid Dosage: Allen (Ex. 1018) and dosages within 350-600 µg



US005563126A

United States Patent [19]
Allen et al.

[11] **Patent Number: 5,563,126**
[45] **Date of Patent: * Oct. 8, 1996**

[54] **METHOD FOR TREATMENT AND PREVENTION OF DEFICIENCIES OF VITAMINS B_{12}, FOLIC ACID, AND B_6**

[75] Inventors: **Robert H. Allen**, Englewood; **Sally P. Stabler**, Denver, both of Colo.

[73] Assignee: **Metabolite Laboratories**, Denver, Colo.

[*] Notice: The portion of the term of this patent subsequent to Dec. 20, 2011, has been disclaimed.

[21] Appl. No.: **999,499**

[22] Filed: **Dec. 29, 1992**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 727,628, Jul. 10, 1991, Pat. No. 5,374,560, which is a continuation-in-part of Ser. No. 333,124, Apr. 3, 1989, abandoned, and Ser. No. 345,885, May 1, 1989, abandoned, which is a continuation-in-part of Ser. No. 933,553, Nov. 20, 1986, Pat. No. 4,940,658.

[51] **Int. Cl.**[6] **A61K 31/70**; A61K 31/495; A61K 31/44

[52] **U.S. Cl.** **514/52**; 514/249; 514/345

[58] **Field of Search** 514/52, 249, 345, 514/814

[56] **References Cited**

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Barness, American Journ. of Clin. Nutr. (20) 1967 pp. 573–577.

Primary Examiner—Raymond Henley, III
Attorney, Agent, or Firm—Davis, Graham & Stubbs, L.L.C.

[57] **ABSTRACT**

A method for orally administering vitamin preparations is described which combine vitamin B_{12} (B_{12}, cobalamin) and folic acid (folate), with and without pyridoxine (B_6), for preventing and treating elevated serum homocysteine (HC), cystathionine (CT), methylmalonic acid (MMA), or 2-methylcitric acid (2-MCA) levels. These metabolites have been shown to be indicative of B_{12} and/or folic acid deficiencies. Further, it is likely that a B_6 deficiency may be present with a B_{12} or folate deficiency. The method of the invention is also for use in lowering serum HC, CT, MMA, or 2-MCA in patients with or at risk for neuropsychiatric, vascular, renal or hematologic diseases. One embodiment of the invention is the use of a non-prescription formulation containing 2.0 mg B_{12} and 0.4 mg folic acid, with and without 25 mg B_6. Another embodiment uses a prescription strength formulation containing 2.0 mg B_{12} and 1.0 mg folic acid, with and without 25 mg B_6. The method of the present invention eliminate the costly and time consuming steps of distinguishing between vitamin deficiencies once a deficiency is found by measurement of serum metabolite levels. The present invention is of particular benefit to the populations at risk for elevated serum metabolite levels, such as the people over the age of 65, and populations that have or are at risk for neuropsychiatric, vascular, renal and hematologic diseases.

10 Claims, 11 Drawing Sheets

Sandoz Inc.
Exhibit 1018-0001

hematologic diseases. One embodiment of the present invention uses a non-prescription formulation comprising between 0.3–10.0 mg B_{12} and 0.1–0.4 mg folate, with the preferred embodiment using 2.0 mg B_{12} and 0.4 mg folate. Another embodiment of the non-prescription formulation uses 0.3–10 mg B_{12}, 0.1–0.4 mg folate, and 5–75 mg B_6, with the preferred embodiment using 2.0 mg B_{12}, 0.4 mg folate, and 25 mg B_6. Another embodiment of the present invention uses a prescription strength formulation comprising between 0.3–10.0 mg B_{12} and 0.4–1.0 mg folate, with the preferred embodiment using 2 mg B_{12} and 1.0 mg folate.

Ex. 1018, U.S. Pat. No. 5,563,126, col. 1, ll. 26-29; Ex. 1004, Schiff ¶ 94.

One embodiment of the present invention is uses an over-the-counter formulation comprised of between 0.3–10 mg CN-cobalamin (B_{12}) and 0.1–0.4 mg folate. Another embodiment of the non-prescription formulation uses 0.3–10 mg B_{12}, 0.1–0.4 folate, and 5–75 mg B_6. Preferred embodiments of the over-the-counter formulation are comprised of about 2.0 mg B_{12} and 0.4 mg folate, and 2.0 mg B_{12}, 0.4 mg folate, and 25 mg B_6, respectively.

Ex. 1018, U.S. Pat. No. 5,563,126, col. 7, ll. 15-17; Ex. 1004, Schiff ¶ 94.

One embodiment of the present invention uses a non-prescription formulation comprised of between about 0.3–10 mg CN-cobalamin (B_{12}) and 0.1–0.4 mg folate. Another embodiment of the present invention uses a non-prescription formulation comprised of between about 0.3–10 mg B_{12}, 0.1–0.4 mg folate, and 5–75 mg B_6. Preferred embodiments of the non-prescription formulation are comprised of about 2.0 mg B_{12} and 0.4 mg folate, and 2.0 mg B_{12}, 0.4 mg folate, and 25 mg B_6, respectively.

Ex. 1018, U.S. Pat. No. 5,563,126, col. 6, ll. 3-7; Ex. 1004, Schiff ¶ 94.

Folic Acid Dosage: testimony concerning dosages within 350-600 µg

Q What's the recommended daily intake for folate?
A 400 micrograms a day.

Ex. 1086, Zeisel Dep. 83:18-20; Ex. 1075, Schiff Reply ¶ 110.

Q. At what level of folic acid was masking understood to occur as of 1999?
A. You know, the number that was thrown around was this milligram, so, of folic acid. So that was the number that was generally used, but there was no dose response clinical data to support a threshold, or anything else, but this was sort of the magic number that was talked about just based on clinical practice. People knowing, well, I gave them this much, and I saw masking type of -- of -- you know, a clinical observation, but there was no rigorous dose response relationship upon which that level was set, but it was viewed again a milligram was something we didn't want to -- to exceed.

Ex. 2137, Stover Dep. 124:13-125:2; *see also* Paper 68, Sur-Reply at 13-14.

Doctor: In those other contexts in which the low dose of between 350 or 1,000 micrograms of folic acid were used in the prior art, was that in the presence of an antifolate?
MS. LYDIGSEN: Objection. Foundation.
THE WITNESS: In some cases, I think those doses were used with lometrexol.
BY MR. PERLMAN:
Q. No. The only clinical study that you've cited for lometrexol is Laohavinij, which had the 5 milligrams a day, as you said, starting a week before, and then there's one patient referred to in the 974 patent, right?
MS. LYDIGSEN: Objection. Foundation. Objection. Mischaracterizes.
BY MR. PERLMAN:
Q. That's all we have.
A. That patient may also have been present in Young and received half a milligram to one milligram a day of folic acid. And regardless of what attribution we make to -- you know, to that patient, that's another significant piece of data, one reason being that it is in line with the community standard for folic acid dose and schedule.

Ex. 2136, Schiff Dep. Tr. 213:10-213:13.; Paper 70, p.5

Folic Acid Dosage: Non Prescription Physician's Desk Reference ("PDR") (Ex. 1106) and dosages within 350-600 µg

CANCER, NUTRIENTS DEFICIENCY SECONDARY TO

Cancer may be treated with chemotherapeutic agents. The following products may be recommended for relief of nutrients deficiency:

NOVARTIS/843

Amount Per Tablet	% Daily Value for Children 2–4 Years of Age	% Daily Value for Adults and Children 4 or More Years of Age
Vitamin A 5000 I.U.	100%	100%
Vitamin C 60 mg	80%	100%
Vitamin D 400 I.U.	50%	100%
Vitamin E 30 I.U.	150%	100%
Vitamin K 10 mcg	*	*
Thiamin 1.5 mg	110%	100%
Riboflavin 1.7 mg	110%	100%
Niacin 20 mg	110%	100%
Vitamin B_6 2 mg	140%	100%
Folate 0.4 mg	100%	100%
Vitamin B_{12}	100%	100%

Folate 0.4 mg
Vitamin B_{12} 6 mcg

Ex. 1106, Non-Prescription PDR, 403, 843; Ex. 1075, Schiff Reply, ¶ 95

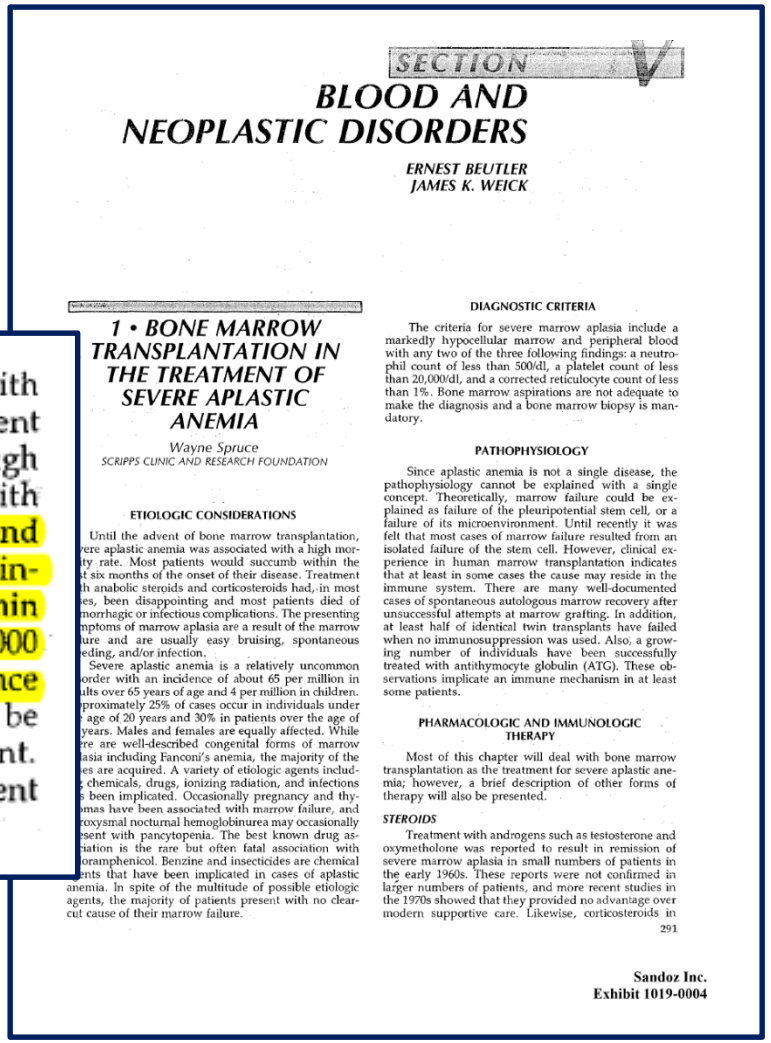
Prior Art Disclosures

STANDARD DOSES OF VITAMIN B_{12}

Prior Art: Beutler (Ex. 1019)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection

Beutler (hematologic disorders)

Patients should be advised that treatment with vitamin B_{12} is to continue for life and that treatment must continue even when they feel better. Although satisfactory maintenance therapy can be achieved with 100 µg of vitamin B_{12} once a month, I tend to recommend 1000 µg each month. Two successful alternative maintenance programs are 1000 µg of hydroxocobalamin every two or three months and eight injections of 1000 µg of hydroxocobalamin over two to three weeks once a year. I suspect a monthly injection is less likely to be forgotten and may be a more reliable form of treatment. I would, therefore, advise as maintenance treatment 1000 µg of vitamin B_{12} once a month for life.



SECTION V

BLOOD AND NEOPLASTIC DISORDERS

ERNEST BEUTLER
JAMES K. WEICK

1 • BONE MARROW TRANSPLANTATION IN THE TREATMENT OF SEVERE APLASTIC ANEMIA

Wayne Spruce
SCRIPPS CLINIC AND RESEARCH FOUNDATION

ETIOLOGIC CONSIDERATIONS

Until the advent of bone marrow transplantation, ere aplastic anemia was associated with a high mor- ity rate. Most patients would succumb within the t six months of the onset of their disease. Treatment h anabolic steroids and corticosteroids had, in most es, been disappointing and most patients died of morrhagic or infectious complications. The presenting mptoms of marrow aplasia are a result of the marrow ure and are usually easy bruising, spontaneous eding, and/or infection.

Severe aplastic anemia is a relatively uncommon order with an incidence of about 65 per million in ults over 65 years of age and 4 per million in children. pproximately 25% of cases occur in individuals under age of 20 years and 30% in patients over the age of years. Males and females are equally affected. While re are well-described congenital forms of marrow asia including Fanconi's anemia, the majority of the es are acquired. A variety of etiologic agents includ- chemicals, drugs, ionizing radiation, and infections s been implicated. Occasionally pregnancy and thy- mas have been associated with marrow failure, and roxysmal nocturnal hemoglobinurea may occasionally sent with pancytopenia. The best known drug as- iation is the rare but often fatal association with oramphenicol. Benzine and insecticides are chemical nts that have been implicated in cases of aplastic anemia. In spite of the multitude of possible etiologic agents, the majority of patients present with no clear- cut cause of their marrow failure.

DIAGNOSTIC CRITERIA

The criteria for severe marrow aplasia include a markedly hypocellular marrow and peripheral blood with any two of the three following findings: a neutrophil count of less than 500/dl, a platelet count of less than 20,000/dl, and a corrected reticulocyte count of less than 1%. Bone marrow aspirations are not adequate to make the diagnosis and a bone marrow biopsy is mandatory.

PATHOPHYSIOLOGY

Since aplastic anemia is not a single disease, the pathophysiology cannot be explained with a single concept. Theoretically, marrow failure could be explained as failure of the pleuripotential stem cell, or a failure of its microenvironment. Until recently it was felt that most cases of marrow failure resulted from an isolated failure of the stem cell. However, clinical experience in human marrow transplantation indicates that at least in some cases the cause may reside in the immune system. There are many well-documented cases of spontaneous autologous marrow recovery after unsuccessful attempts at marrow grafting. In addition, at least half of identical twin transplants have failed when no immunosuppression was used. Also, a growing number of individuals have been successfully treated with antithymocyte globulin (ATG). These observations implicate an immune mechanism in at least some patients.

PHARMACOLOGIC AND IMMUNOLOGIC THERAPY

Most of this chapter will deal with bone marrow transplantation as the treatment for severe aplastic anemia; however, a brief description of other forms of therapy will also be presented.

STEROIDS

Treatment with androgens such as testosterone and oxymetholone was reported to result in remission of severe marrow aplasia in small numbers of patients in the early 1960s. These reports were not confirmed in larger numbers of patients, and more recent studies in the 1970s showed that they provided no advantage over modern supportive care. Likewise, corticosteroids in

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Exhibit 1019-0004

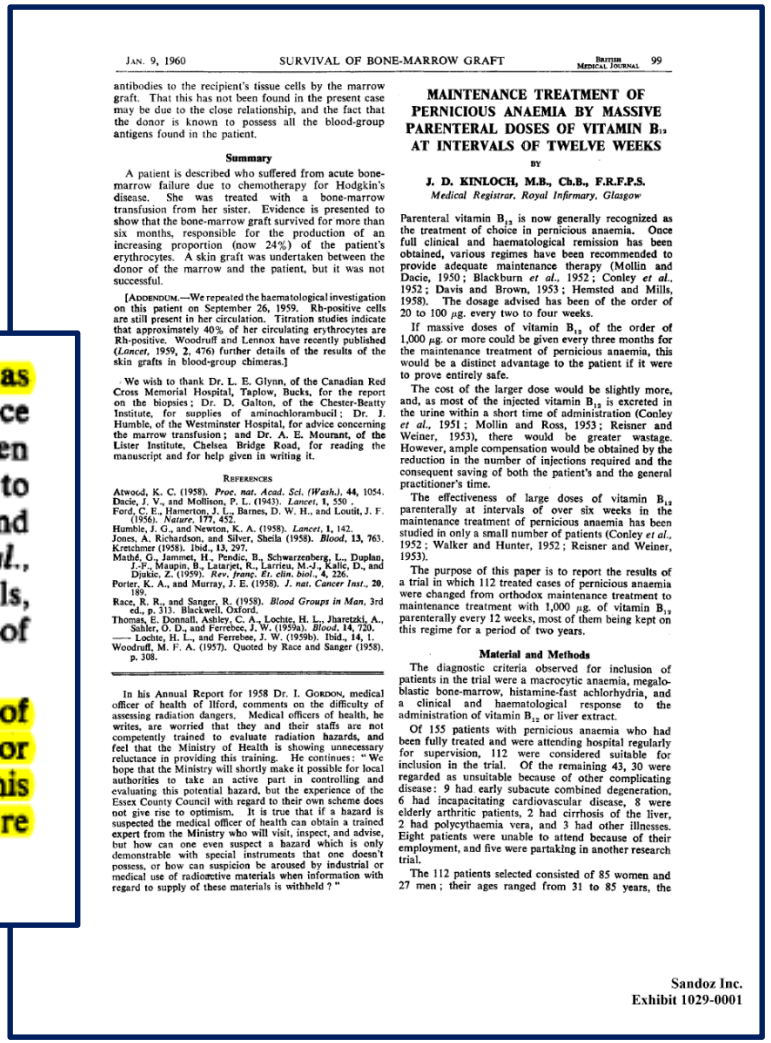
Ex. 1019, Beutler at 302; Ex. 1004, Schiff Decl. ¶102; Ex. 1075, Schiff Reply, ¶112

Prior Art: Kinloch (Ex. 1029)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection

Kinloch (anaemia)

Parenteral vitamin B_{12} is now generally recognized as the treatment of choice in pernicious anaemia. Once full clinical and haematological remission has been obtained, various regimes have been recommended to provide adequate maintenance therapy (Mollin and Dacie, 1950; Blackburn *et al.*, 1952; Conley *et al.*, 1952; Davis and Brown, 1953; Hemsted and Mills, 1958). The dosage advised has been of the order of 20 to 100 µg. every two to four weeks.

If massive doses of vitamin B_{12} of the order of 1,000 µg. or more could be given every three months for the maintenance treatment of pernicious anaemia, this would be a distinct advantage to the patient if it were to prove entirely safe.



JAN. 9, 1960 SURVIVAL OF BONE-MARROW GRAFT BRITISH MEDICAL JOURNAL 99

antibodies to the recipient's tissue cells by the marrow graft. That this has not been found in the present case may be due to the close relationship, and the fact that the donor is known to possess all the blood-group antigens found in the patient.

Summary

A patient is described who suffered from acute bone-marrow failure due to chemotherapy for Hodgkin's disease. She was treated with a bone-marrow transfusion from her sister. Evidence is presented to show that the bone-marrow graft survived for more than six months, responsible for the production of an increasing proportion (now 24%) of the patient's erythrocytes. A skin graft was undertaken between the donor of the marrow and the patient, but it was not successful.

[ADDENDUM.—We repeated the haematological investigation on this patient on September 26, 1959. Rh-positive cells are still present in her circulation. Titration studies indicate that approximately 40% of her circulating erythrocytes are Rh-positive. Woodruff and Lennox have recently published (*Lancet*, 1959, **2**, 476) further details of the results of the skin grafts in blood-group chimeras.]

We wish to thank Dr. L. E. Glynn, of the Canadian Red Cross Memorial Hospital, Taplow, Bucks, for the report on the biopsies; Dr. D. Galton, of the Chester-Beatty Institute, for supplies of aminochlorambucil; Dr. J. Humble, of the Westminster Hospital, for advice concerning the marrow transfusion; and Dr. A. E. Mourant, of the Lister Institute, Chelsea Bridge Road, for reading the manuscript and for help given in writing it.

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—— Lochte, H. L., and Ferrebee, J. W. (1959b). Ibid., **14**, 1.
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In his Annual Report for 1958 Dr. I. GORDON, medical officer of health of Ilford, comments on the difficulty of assessing radiation dangers. Medical officers of health, he writes, are worried that they and their staffs are not competently trained to evaluate radiation hazards, and feel that the Ministry of Health is showing unnecessary reluctance in providing this training. He continues: "We hope that the Ministry will shortly make it possible for local authorities to take an active part in controlling and evaluating this potential hazard, but the experience of the Essex County Council with regard to their own scheme does not give rise to optimism. It is true that if a hazard is suspected the medical officer of health can obtain a trained expert from the Ministry who will visit, inspect, and advise, but how can one even suspect a hazard which is only demonstrable with special instruments that one doesn't possess, or how can suspicion be aroused by industrial or medical use of radioactive materials when information with regard to supply of these materials is withheld?"

MAINTENANCE TREATMENT OF PERNICIOUS ANAEMIA BY MASSIVE PARENTERAL DOSES OF VITAMIN B_{12} AT INTERVALS OF TWELVE WEEKS

BY

J. D. KINLOCH, M.B., Ch.B., F.R.F.P.S.

Medical Registrar, Royal Infirmary, Glasgow

Parenteral vitamin B_{12} is now generally recognized as the treatment of choice in pernicious anaemia. Once full clinical and haematological remission has been obtained, various regimes have been recommended to provide adequate maintenance therapy (Mollin and Dacie, 1950; Blackburn *et al.*, 1952; Conley *et al.*, 1952; Davis and Brown, 1953; Hemsted and Mills, 1958). The dosage advised has been of the order of 20 to 100 µg. every two to four weeks.

If massive doses of vitamin B_{12} of the order of 1,000 µg. or more could be given every three months for the maintenance treatment of pernicious anaemia, this would be a distinct advantage to the patient if it were to prove entirely safe.

The cost of the larger dose would be slightly more, and, as most of the injected vitamin B_{12} is excreted in the urine within a short time of administration (Conley *et al.*, 1951; Mollin and Ross, 1953; Reisner and Weiner, 1953), there would be greater wastage. However, ample compensation would be obtained by the reduction in the number of injections required and the consequent saving of both the patient's and the general practitioner's time.

The effectiveness of large doses of vitamin B_{12} parenterally at intervals of over six weeks in the maintenance treatment of pernicious anaemia has been studied in only a small number of patients (Conley *et al.*, 1952; Walker and Hunter, 1952; Reisner and Weiner, 1953).

The purpose of this paper is to report the results of a trial in which 112 treated cases of pernicious anaemia were changed from orthodox maintenance treatment to maintenance treatment with 1,000 µg. of vitamin B_{12} parenterally every 12 weeks, most of them being kept on this regime for a period of two years.

Material and Methods

The diagnostic criteria observed for inclusion of patients in the trial were a macrocytic anaemia, megaloblastic bone-marrow, histamine-fast achlorhydria, and a clinical and haematological response to the administration of vitamin B_{12} or liver extract.

Of 155 patients with pernicious anaemia who had been fully treated and were attending hospital regularly for supervision, 112 were considered suitable for inclusion in the trial. Of the remaining 43, 30 were regarded as unsuitable because of other complicating disease: 9 had early subacute combined degeneration, 6 had incapacitating cardiovascular disease, 8 were elderly arthritic patients, 2 had cirrhosis of the liver, 2 had polycythaemia vera, and 3 had other illnesses. Eight patients were unable to attend because of their employment, and five were partaking in another research trial.

The 112 patients selected consisted of 85 women and 27 men; their ages ranged from 31 to 85 years, the

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Exhibit 1029-0001

Ex. 1029, Kinloch at 99; Ex. 1004, Schiff Decl. ¶103; Ex. 1075, Schiff Reply, 112 ¶

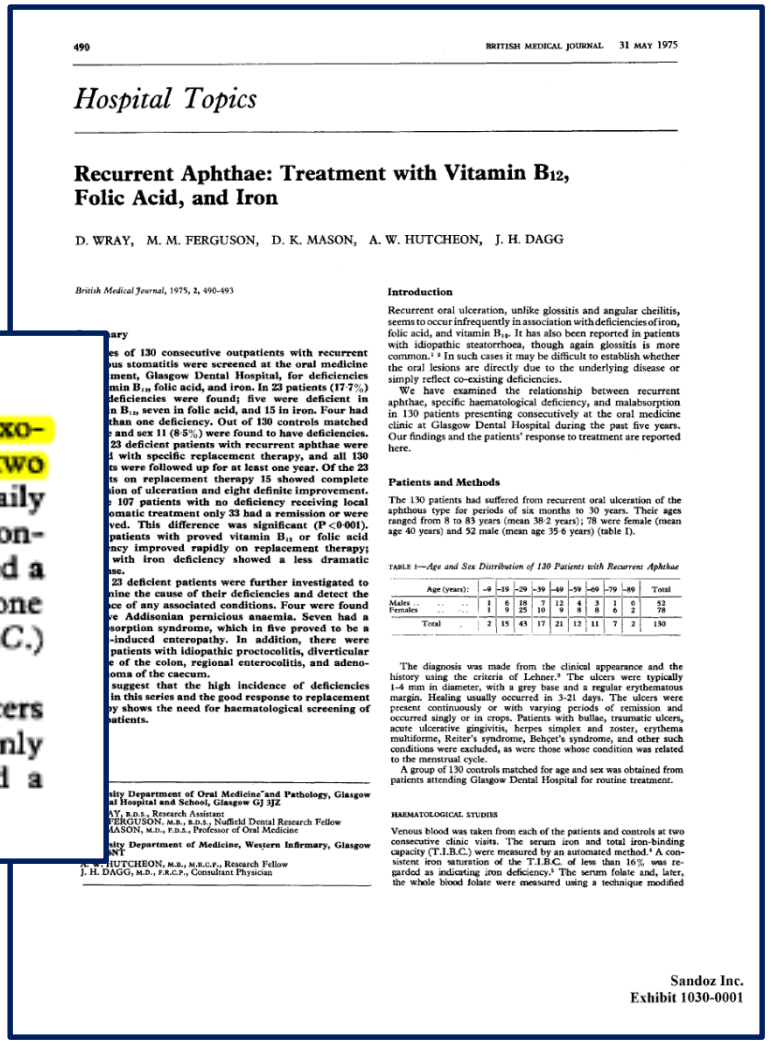
Prior Art: Wray (Ex. 1030)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection

Wray (apthae ulcers)

TREATMENT

Patients with vitamin B_{12} deficiency were given 1000 µg hydroxocobalamin intramuscularly followed by a further 1000 µg every two months. Folic acid was taken by mouth in doses of 5 mg thrice daily during follow-up. Iron was also taken only by mouth and given continuously for at least six months. During treatment all patients used a zinc chloride/zinc sulphate mouthwash (*B.P.C.*). Triamcinolone 0·1% in dental paste (*B.P.C.*) or hydrocortisone lozenges (*B.P.C.*) were also used for symptomatic relief.

In assessing the response to treatment, complete absence of ulcers for at least one year after treatment constituted a remission, and only occasional ulcers after treatment (one to six a year) constituted a definite improvement.



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Hospital Topics

Recurrent Aphthae: Treatment with Vitamin B_{12}, Folic Acid, and Iron

D. WRAY, M. M. FERGUSON, D. K. MASON, A. W. HUTCHEON, J. H. DAGG

British Medical Journal, 1975, 2, 490-493

Introduction

Recurrent oral ulceration, unlike glossitis and angular cheilitis, seems to occur infrequently in association with deficiencies of iron, folic acid, and vitamin B_{12}. It has also been reported in patients with idiopathic steatorrhoea, though again glossitis is more common.[1 2] In such cases it may be difficult to establish whether the oral lesions are directly due to the underlying disease or simply reflect co-existing deficiencies.

We have examined the relationship between recurrent aphthae, specific haematological deficiency, and malabsorption in 130 patients presenting consecutively at the oral medicine clinic at Glasgow Dental Hospital during the past five years. Our findings and the patients' response to treatment are reported here.

Patients and Methods

The 130 patients had suffered from recurrent oral ulceration of the aphthous type for periods of six months to 30 years. Their ages ranged from 8 to 83 years (mean 38·2 years); 78 were female (mean age 40 years) and 52 male (mean age 35·6 years) (table I).

TABLE I—*Age and Sex Distribution of 130 Patients with Recurrent Aphthae*

Age (years):	-9	-19	-29	-39	-49	-59	-69	-79	-89	Total
Males	1	6	18	7	12	4	3	1	0	52
Females	1	9	25	10	9	8	6	6	2	78
Total	2	15	43	17	21	12	11	7	2	130

The diagnosis was made from the clinical appearance and the history using the criteria of Lehner.[3] The ulcers were typically 1-4 mm in diameter, with a grey base and a regular erythematous margin. Healing usually occurred in 3-21 days. The ulcers were present continuously or with varying periods of remission and occurred singly or in crops. Patients with bullae, traumatic ulcers, acute ulcerative gingivitis, herpes simplex and zoster, erythema multiforme, Reiter's syndrome, Behçet's syndrome, and other such conditions were excluded, as were those whose condition was related to the menstrual cycle.

A group of 130 controls matched for age and sex was obtained from patients attending Glasgow Dental Hospital for routine treatment.

HAEMATOLOGICAL STUDIES

Venous blood was taken from each of the patients and controls at two consecutive clinic visits. The serum iron and total iron-binding capacity (T.I.B.C.) were measured by an automated method.[4] A consistent iron saturation of the T.I.B.C. of less than 16% was regarded as indicating iron deficiency.[5] The serum folate and, later, the whole blood folate were measured using a technique modified

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Exhibit 1030-0001

Ex. 1030, Wray at 491; Ex. 1004, Schiff Decl. ¶104; Ex. 1075, Schiff Reply, ¶112

Prior Art: Tamura (Ex. 1031)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection

Tamura (immunological, neurological, and oncological disorders)

Study design

Leucocyte and lymphocyte numbers, percentage and absolute numbers of $CD4^+$ cells and $CD8^+$ cells, CD4/CD8 ratio and NK cell activity were evaluated in all patients at diagnosis and compared with the values in control subjects.

In order to examine the immunomodulatory effect of vit.B12, methylcobalamin was administrated to all patients and to eight of 13 volunteers as follows. Methyl-vit.B12 (500 µg/day; methyl-B12; mecobalamin; Eisai, Tokyo, Japan) was injected intramuscularly every other day for 2 weeks and immunophenotyping of peripheral lymphocytes and NK cell activity were evaluated as before treatment. At that time, all patients and control subjects showed high serum levels of vit.B12 (>3000 pg/ml). After 2 weeks treatment, patients were treated with vit.B12 1000 µg every 3 months as out-patients; all of them were quite well and anaemia had improved. After 1–2 years of follow up, NK cell activity was estimated in seven of 11 patients who showed high serum levels of vit.B12 (>3000 pg/ml).

Clin Exp Immunol 1999; 116:28–32

Immunomodulation by vitamin B12: augmentation of $CD8^+$ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment

J. TAMURA, K. KUBOTA*, H. MURAKAMI†, M. SAWAMURA, T. MATSUSHIMA, T. TAMURA, T. SAITOH, H. KURABAYSHI* & T. NARUSE *Third Department of Internal Medicine, Gunma University School of Medicine, Maebashi, *Department of Internal Medicine, Kusatsu Branch Hospital, Gunma University Hospital, Kusatsu, and †School of Health Sciences, Gunma University, Maebashi, Japan*

(Accepted for publication 7 January 1999)

SUMMARY

It has been suggested that vitamin B12 (vit.B12) plays an important role in immune system regulation, but the details are still obscure. In order to examine the action of vit.B12 on cells of the human immune system, lymphocyte subpopulations and NK cell activity were evaluated in 11 patients with vit.B12 deficiency anaemia and in 13 control subjects. Decreases in the number of lymphocytes and $CD8^+$ cells and in the proportion of $CD4^+$ cells, an abnormally high CD4/CD8 ratio, and suppressed NK cell activity were noted in patients compared with control subjects. In all 11 patients and eight control subjects, these immune parameters were evaluated before and after methyl-B12 injection. The lymphocyte counts and number of $CD8^+$ cells increased both in patients and in control subjects. The high CD4/CD8 ratio and suppressed NK cell activity were improved by methyl-B12 treatment. Augmentation of $CD3^-CD16^+$ cells occurred in patients after methyl-B12 treatment. In contrast, antibody-dependent cell-mediated cytotoxicity (ADCC) activity, lectin-stimulated lymphocyte blast formation, and serum levels of immunoglobulins were not changed by methyl-B12 treatment. These results indicate that vit.B12 might play an important role in cellular immunity, especially relativing to $CD8^+$ cells and the NK cell system, which suggests effects on cytotoxic cells. We conclude that vit.B12 acts as an immunomodulator for cellular immunity.

Keywords vitamin B12 NK cell CD8 immunomodulation

INTRODUCTION

B12 (Vit.B12) has various effects on biological processes is well known that megaloblastic anaemia and peripheral urbances are caused by lack of vit.B12. In the immune n important role of vit.B12 has been reported. Vit.B12 T cell proliferative responses to concanavalin A (Con A) unoglobulin synthesis of B cells by pokeweed mitogen 1]. It has been reported that vit.B12 deficiency caused n of protective immune responses to viruses and bacteria nal model [2].

man immunity, the action of vit.B12 is still obscure, because it is impossible to study the action of vit.B12 ficially deficient human model systems. However, we lly encounter patients with vit.B12 deficiency disorders, such as megaloblastic anaemia, and it is possible to observe the change of immunological parameters after vit.B12 administration in such patients. A few investigations and case reports of immunological abnormalities in vit.B12-deficient megaloblastic anaemia patients have been reported [3–7], but there have been no systematic studies.

In order to investigate the biological actions of vit.B12 on cells of the human immune system, lymphocyte subsets and NK cell activities were examined in patients with vit.B12 deficiency anaemia, and the changes of immune parameters after vit.B12 administration were evaluated.

pondence: J. Tamura, Third Department of Internal Medicine, niversity School of Medicine, 3 Showa-machi, Maebashi Japan.

SUBJECTS AND METHODS

Subjects

Eleven newly diagnosed Japanese patients with vit.B12 deficiency anaemia were admitted to our hospital between December 1990

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Exhibit 1031-0001

Ex. 1031, Tamura at 29; Ex. 1004, Schiff Decl. ¶105

Prior Art: Tamura (Ex. 1031)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection

Tamura (immunological, neurological, and oncological disorders)

In conclusion, we found a significant decrease in the absolute number of $CD8^+$ cells and suppressed NK cell activity in vit.B12-deficient patients. These abnormalities could be at least partly restored by methyl-B12 treatment. Moreover, augmentation of $CD8^+$ cells by methyl-B12 treatment was observed even in control subjects. These observations may contribute to our understanding of the potential anti-tumour effects of vit.B12, and may partly explain the high risk of gastric carcinoma in PA; our data also provide a rationale for considering the use of vit.B12 for treating a variety of other immunological, neurological, and oncological disorders.

Clin Exp Immunol 1999; 116:28–32

Immunomodulation by vitamin B12: augmentation of $CD8^+$ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment

J. TAMURA, K. KUBOTA*, H. MURAKAMI†, M. SAWAMURA, T. MATSUSHIMA, T. TAMURA, T. SAITOH, H. KURABAYSHI* & T. NARUSE *Third Department of Internal Medicine, Gunma University School of Medicine, Maebashi, *Department of Internal Medicine, Kusatsu Branch Hospital, Gunma University Hospital, Kusatsu, and †School of Health Sciences, Gunma University, Maebashi, Japan*

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Keywords vitamin B12 NK cell CD8 immunomodulation

INTRODUCTION

B12 (Vit.B12) has various effects on biological processes is well known that megaloblastic anaemia and peripheral urbances are caused by lack of vit.B12. In the immune n important role of vit.B12 has been reported. Vit.B12 T cell proliferative responses to concanavalin A (Con A) imoglobulin synthesis of B cells by pokeweed mitogen (PWM) [1]. It has been reported that vit.B12 deficiency caused suppression of protective immune responses to viruses and bacteria in an animal model [2].

In human immunity, the action of vit.B12 is still obscure, probably because it is impossible to study the action of vit.B12 using artificially deficient human model systems. However, we occasionally encounter patients with vit.B12 deficiency disorders, such as megaloblastic anaemia, and it is possible to observe the change of immunological parameters after vit.B12 administration in such patients. A few investigations and case reports of immunological abnormalities in vit.B12-deficient megaloblastic anaemia patients have been reported [3–7], but there have been no systematic studies.

In order to investigate the biological actions of vit.B12 on cells of the human immune system, lymphocyte subsets and NK cell activities were examined in patients with vit.B12 deficiency anaemia, and the changes of immune parameters after vit.B12 administration were evaluated.

Correspondence: J. Tamura, Third Department of Internal Medicine, Gunma University School of Medicine, 3 Showa-machi, Maebashi 371-8511, Japan.

SUBJECTS AND METHODS

Subjects

Eleven newly diagnosed Japanese patients with vit.B12 deficiency anaemia were admitted to our hospital between December 1990

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Exhibit 1031-0001

Ex. 1031, Tamura at 31; Ex. 1004, Schiff Decl. ¶105; Ex. 1075, Schiff Reply ¶112

Prior Art: Clarke (Ex. 2070)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection

Clarke

Our results suggest that a daily dose of at least 0.5 mg of folic acid, along with a similar amount of vitamin B-12, would produce a proportional reduction in blood homocysteine of about a quarter to a third. The addition of about 1 mg daily of oral vitamin B-12 to folic acid would also be expected to avoid the theoretical risk of neuropathy due to unopposed folic acid therapy in patients deficient in vitamin B-12, even those with intrinsic factor deficiency or malabsorption states.[26–28] Studies in the United States and Britain indicate that the average concentration of blood homocysteine in a typical Western population is about 12 µmol/l,[4,9] and so a reduction of about a quarter to a third would correspond to an absolute reduction of about 3-4 µmol/l. A previous meta-analysis of the observational studies suggests that a prolonged lower blood homocysteine concentration of 3-4 µmol/l would correspond to 30-40% less vascular disease.[1] Consequently, even if as much as half of the epidemiologically predicted benefit is achieved within a few years of lowering blood homocysteine (as seems to be the case with cholesterol lowering[28–31]), trials of folic acid supplements may well need to be large, and to include people at high risk, to be able to detect the sort of reductions—15% to 20%—in cardiovascular risk that might realistically be anticipated.

Papers

minute. Airway hypoxia was discontinued as soon as possible in each infant who showed this degree of desaturation; it should be remembered that this required the tent to be opened and the gas mixture to be removed from around the baby. No infant remained at ≤80% in 15% oxygen for longer than 126 seconds.

Of the four infants in whom exposure to hypoxia was discontinued early, one infant had a sibling who had died of the sudden infant death syndrome and was already being monitored at home. Oxygen saturation levels in all four infants remained within the normal range during subsequent monitoring. We believed that monitoring the infants for a longer period in hospital would not have been ethically appropriate because they might be exposed to additional risks (for example, the risk of acquiring an infection in hospital). The two infants who had died following an aircraft flight were not monitored so we are unaware of the duration and degree of hypoxaemia to which they might have been exposed.

Although Savulescu's commentary raises the spectre of human or mechanical error, we took every precaution to ensure that the infants were safe. These included the use of a special medical gas mixture of 15% oxygen and 85% nitrogen instead of air diluted with nitrogen, continuous monitoring of the partial pressure of inspired carbon dioxide to identify rebreathing, and continuous monitoring of the partial pressure of inspired oxygen to ensure adequate ventilation of the tent with the gas mixture. The study was done in a room near the intensive care unit. There was also continuous surveillance by an experienced paediatrician of the readings from the pulse oximeter, transcutaneous monitoring of the partial pressure of carbon dioxide, monitoring of respiratory movement, and electrocardiography.

Although Milner reports in his editorial that British Airways identified no deaths on the undisclosed number of flights involving infants, this is low quality information. It is not accurate, as shown by the personal communication cited in our paper. Infant stimulation and the attention paid to an infant during an airline flight may delay potentially serious consequences of the flight until after the plane's arrival. British Airways would not have access to information on infants after arrival and did not seem to know about either of the two cases of the sudden infant death syndrome that were described in our report.

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Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials

Homocysteine Lowering Trialists' Collaboration

Participants in the collaboration are listed at the end of the paper

Correspondence to: Dr Robert Clarke, Homocysteine Lowering Trialists' Collaboration, Clinical Trial Service Unit, Radcliffe Infirmary, Oxford OX2 6HE. robert.clarke@ctsu.ox.ac.uk

BMJ 1998;316:894–8

Abstract

Objective: To determine the size of reduction in homocysteine concentrations produced by dietary supplementation with folic acid and with vitamins B-12 or B-6.

Design: Meta-analysis of randomised controlled trials that assessed the effects of folic acid based supplements on blood homocysteine concentrations. Multivariate regression analysis was used to determine the effects on homocysteine concentrations of different doses of folic acid and of the addition of vitamin B-12 or B-6.

Subjects: Individual data on 1114 people included in 12 trials.

Findings: The proportional and absolute reductions in blood homocysteine produced by folic acid supplements were greater at higher pretreatment blood homocysteine concentrations ($P < 0.001$) and at lower pretreatment blood folate concentrations ($P < 0.001$). After standardisation to pretreatment blood concentrations of homocysteine of 12 µmol/l and of folate of 12 nmol/l (approximate average concentrations for Western populations), dietary folic acid reduced blood homocysteine concentrations by 25% (95% confidence interval 23% to 28%; $P < 0.001$), with similar effects in the range of 0.5-5 mg folic acid daily. Vitamin B-12 (mean 0.5 mg daily) produced an additional 7% (5% to 10%) reduction in blood homocysteine. Vitamin B-6 (mean 16.5 mg daily) did not have a significant additional effect.

Conclusions: Typically in Western populations, daily supplementation with both 0.5-5 mg folic acid and about 0.5 mg vitamin B-12 would be expected to reduce blood homocysteine concentrations by about a quarter to a third (for example, from about 12 µmol/l to 8-9 µmol/l). Large scale randomised trials of such regimens in high risk populations are now needed to determine whether lowering blood homocysteine concentrations reduces the risk of vascular disease.

Introduction

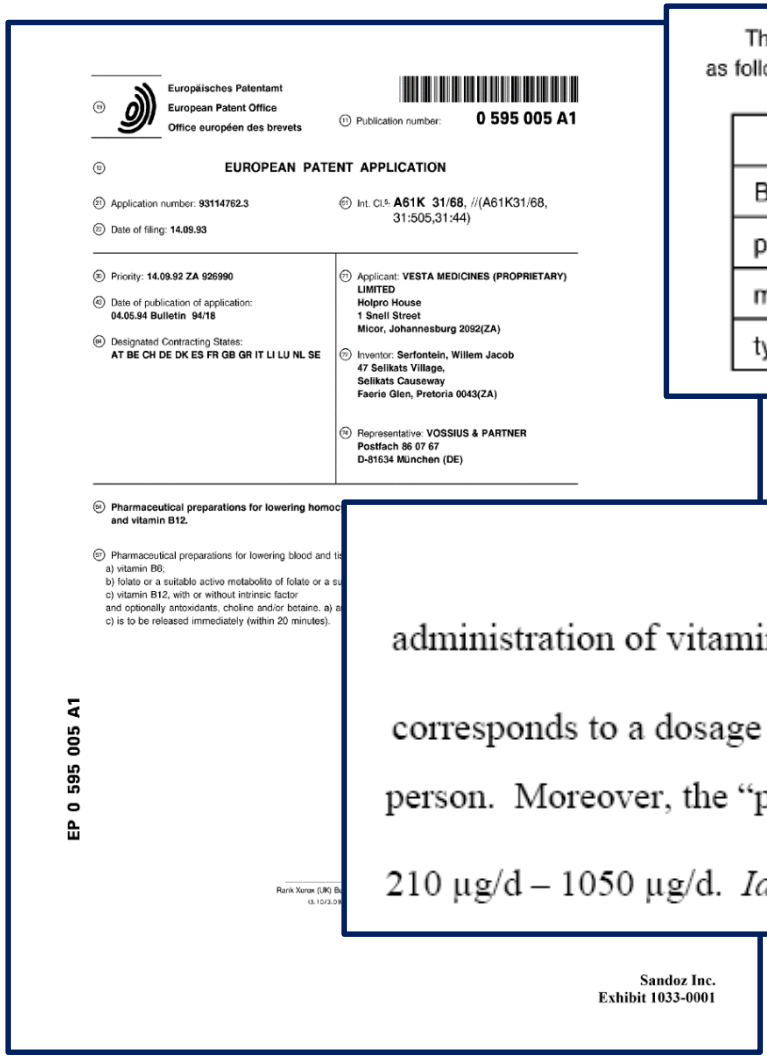
Epidemiological studies have consistently reported that patients with occlusive vascular disease have higher blood homocysteine concentrations than

Lilly Ex. 2070
Sandoz v. Lilly IPR2016-00318

Ex. 2070, Clarke at 897; Ex. 1075, Schiff Reply ¶115

Sandoz DX -106

Prior Art: EP005 (Ex. 1033)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection



Europäisches Patentamt
European Patent Office
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(11) Publication number: **0 595 005 A1**

EUROPEAN PATENT APPLICATION

(21) Application number: 93114762.3
(22) Date of filing: 14.09.93
(51) Int. Cl.5: A61K 31/68, //(A61K31/68, 31:505,31:44)

(30) Priority: 14.09.92 ZA 926990
(43) Date of publication of application: 04.05.94 Bulletin 94/18
(84) Designated Contracting States: AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(71) Applicant: VESTA MEDICINES (PROPRIETARY) LIMITED
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(54) Pharmaceutical preparations for lowering homoc
and vitamin B12.

(57) Pharmaceutical preparations for lowering blood and ti
a) vitamin B6;
b) folate or a suitable active metabolite of folate or a su
c) vitamin B12, with or without intrinsic factor
and optionally antioxidants, choline and/or betaine. a)
c) is to be released immediately (within 20 minutes).

EP 0 595 005 A1

Rank Xerox (UK) B

Sandoz Inc.
Exhibit 1033-0001

The preparations in accordance with the invention are formulated to provide approximate daily dosages as follows (µg/d/kg body weight).

	a) Vitamin B6	b) Folic Acid	c) Vitamin B12
Broadest range	15-750	1,5-150	1,5-75
preferred range	30-400	7,5-50	3-15
more preferred range	75-250	10-30	7-10
typical example	150	15	7,5

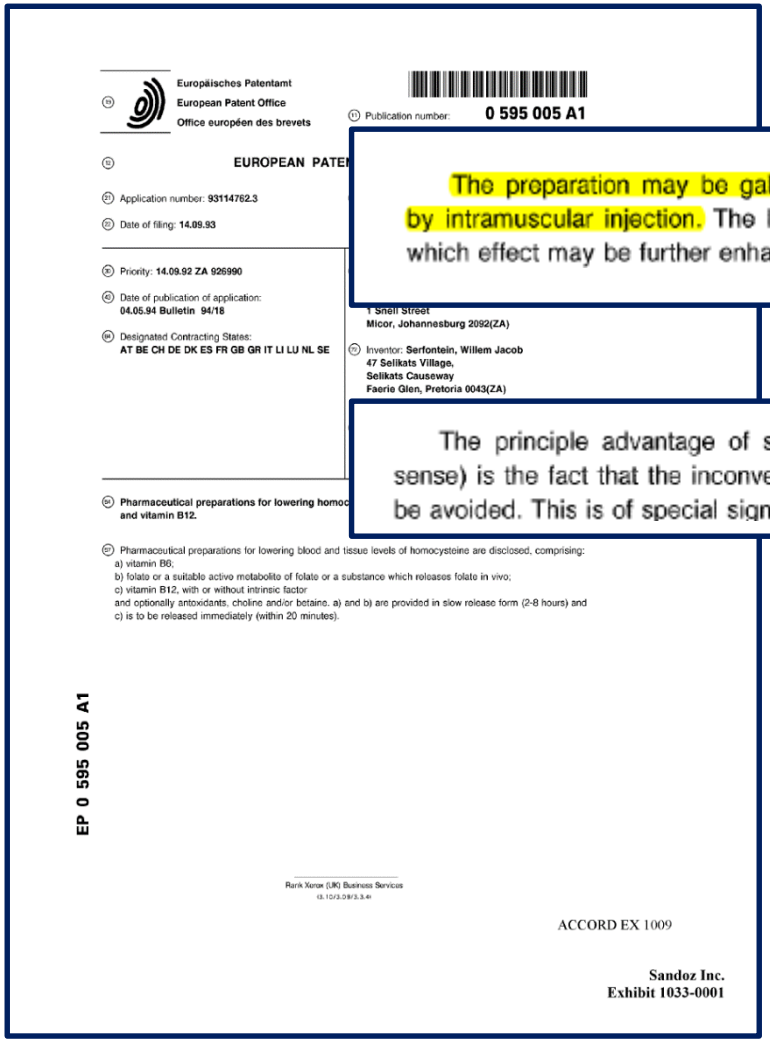
Ex. 1033, EP 005 at 4-5, 9; Ex. 1004, Schiff Decl. ¶107

EP005 discloses

administration of vitamin B_{12} in a range of 1.5-75 µg/d/kg body weight, which corresponds to a dosage of approximately 105 µg/d – 5,250 µg/d for a 70 kg person. Moreover, the "preferred" range of 3-15 µg/d/kg corresponds to a range of 210 µg/d – 1050 µg/d. *Id.* at 5.

Ex. 1004, Schiff Decl. ¶107

Prior Art: EP005 (Ex. 1033)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection



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(11) Publication number: **0 595 005 A1**

EUROPEAN PATE

(21) Application number: 93114762.3

(22) Date of filing: 14.09.93

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(84) Designated Contracting States:
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(72) Inventor: Serfontein, Willem Jacob
47 Selikats Village,
Selikats Causeway
Faerie Glen, Pretoria 0043(ZA)

(54) Pharmaceutical preparations for lowering homoc
and vitamin B12.

(57) Pharmaceutical preparations for lowering blood and tissue levels of homocysteine are disclosed, comprising:
a) vitamin B6;
b) folate or a suitable active metabolite of folate or a substance which releases folate in vivo;
c) vitamin B12, with or without intrinsic factor
and optionally antioxidants, choline and/or betaine. a) and b) are provided in slow release form (2-8 hours) and
c) is to be released immediately (within 20 minutes).

EP 0 595 005 A1

Rank Xerox (UK) Business Services

ACCORD EX 1009

Sandoz Inc.
Exhibit 1033-0001

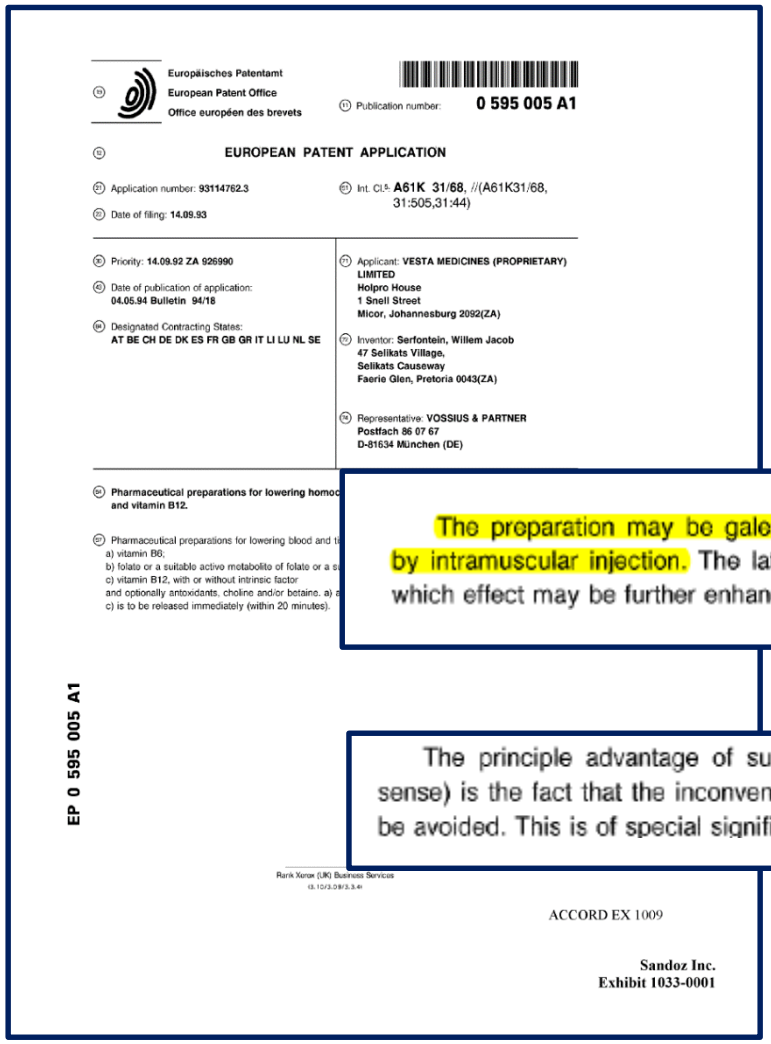
> The preparation may be galenically formulated for parenteral administration, preferably by infusion or by intramuscular injection. The latter form inherently provides for a retarded availability of the ingredients, which effect may be further enhanced by depot forms of formulation.

Ex. 1033, EP 005, 5:52-54; Ex. 1004, Schiff Decl. ¶107

> The principle advantage of such parenteral formulations (applying the term "parenteral" in a broad sense) is the fact that the inconvenient, unpleasant and often costly application by means of injections can be avoided. This is of special significance in the case of vitamin B12 and coenzyme Q10.

Ex. 2120, Chabner Decl. ¶¶214-15 (citing Ex. 1033, EP 005, 7:48-51); Ex. 1004, Schiff Reply ¶116

Prior Art: EP005 (Ex. 1033)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection



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(11) Publication number: **0 595 005 A1**

EUROPEAN PATENT APPLICATION

(21) Application number: 93114762.3
(22) Date of filing: 14.09.93
(51) Int. Cl.5: A61K 31/68, //(A61K31/68, 31:505,31:44)

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EP 0 595 005 A1

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The principle advantage of such parenteral formulations (applying the term "parenteral" in a broad sense) is the fact that the inconvenient, unpleasant and often costly application by means of injections can be avoided. This is of special significance in the case of vitamin B12 and coenzyme Q10.

Ex. 1033, EP 005, 7:48-51; Ex. 1004, Schiff Reply ¶116

Prior Art: EP005 (Ex. 1033)
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection

- Dr. Ron Schiff, M.D., Ph.D.

> Indeed, when I encountered patients with poor nutritional status, it was my practice to administer vitamin B_{12} at a 1000 µg dose intramuscularly.

Ex. 1075, Schiff Reply ¶113

- Dr. Patrick Stover, Ph.D.

> And I think there is an assumption in the statement you just made -- in terms of the -- B12 being innocuous, that refers to there not being a particular highest acceptable dose when administered as a nutritional supplement outside of any possible interactions between vitamin B12 and a particular drug; is that fair?
>
> A. What I'm stating is that there are no -- no known toxicities with administering B12 orally or through intramuscular injection, up to a (mg) a day. It has no adverse consequences, so one doesn't have to worry about overdose of a nutrient or excessive levels of exposure when it comes to B12.
>
> So given the fact that there is not a absolute way to diagnose the deficiency and the fact the treatment in no way can present any risk to the patient, B12 administration is often recommended if there is any concern.

Ex. 2137, Stover Dep. Tr. 105:7-25; Ex. 1004, Schiff Decl. ¶1004; *see* Paper 68, Sur-Reply at 13-14.

Dr. Zeisel's Testimony
Vitamin B_{12} Dosage: 1000 µg Intramuscular Injection

Q -- this particular paper and more concerned with, as a practicing nutritionist, what you believe to be the standard intramuscular dose for vitamin B12.

A It varies. And it would have been -- you need about 4 micrograms. But you would have given a dose that is milligrams, probably at that time, as an intramuscular dose.

So that would be 10 to -- you know, or more times what the normal oral dose would have been, because it's to serve as a depot that they can absorb from for a while.

And so it doesn't really matter how much you give intramuscularly; it's just how long it lasts before they lose it by not being able to reabsorb the amount that they excrete into their gut.

Q What do you mean when you say "it doesn't really matter how much you give intramuscularly"?

A So you only need a few micrograms. That's available from the intramuscular dose.

The problem is, for people who can't absorb it every day, they are secreting B12 into their intestine, and then they can't reabsorb it. So they rapidly run themselves down.

And so when you give an IM dose, you don't have to give it to them daily. You give them something more than the few micrograms they need so that they can draw on that dose that's sitting in the muscle that you stuck it in for a period of time.

And so a standard dose probably, you know, a milligram would have been enough to last them weeks before they run it down. A microgram being a millionth of a gram, and a milligram being a thousandth of a gram.

Q I'm sorry. I didn't catch the last part.

A A milligram is a thousandth of a gram; and a microgram is a millionth of a gram. So a milligram is a lot of micrograms.

Q And that dosing was true in 1999; correct?

A Yes, it would have been about that. I don't remember exactly what preparations you could order off the counter.

Ex. 1086, Zeisel Dep. Tr. 89:14-91:14; *see also* Ex. 1075, Schiff Reply ¶116

Prior Art Disclosures

DOSING SCHEDULES

'209 Patent: Dependent claims set out two pretreatment dosing schedules

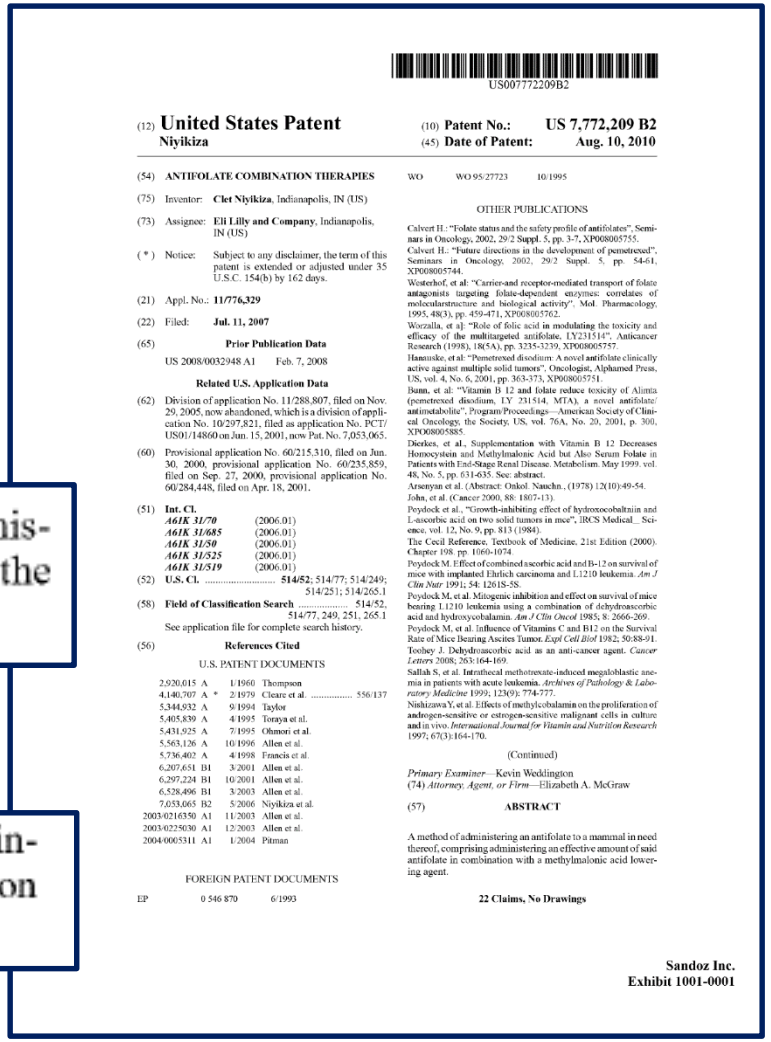
- 1-3 weeks: dependent claims 6 & 19
- 1-24 hours: dependent claims 7 & 20

Claim 19

19. The method of claim **18** wherein folic acid is administered 1 to 3 weeks prior to the first administration of the pemetrexed disodium.

Claim 20

20. The method of claim **18** wherein the folic acid is administered from about 1 to about 24 hours prior to administration of the pemetrexed disodium.



US007772209B2

(12) **United States Patent**
Niyikiza

(10) **Patent No.: US 7,772,209 B2**
(45) **Date of Patent: Aug. 10, 2010**

(54) **ANTIFOLATE COMBINATION THERAPIES**

(75) Inventor: **Clet Niyikiza**, Indianapolis, IN (US)

(73) Assignee: **Eli Lilly and Company**, Indianapolis, IN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 162 days.

(21) Appl. No.: **11/776,329**

(22) Filed: **Jul. 11, 2007**

(65) **Prior Publication Data**

US 2008/0032948 A1 Feb. 7, 2008

Related U.S. Application Data

(62) Division of application No. 11/288,807, filed on Nov. 29, 2005, now abandoned, which is a division of application No. 10/297,821, filed as application No. PCT/US01/14860 on Jun. 15, 2001, now Pat. No. 7,053,065.

(60) Provisional application No. 60/215,310, filed on Jun. 30, 2000, provisional application No. 60/235,859, filed on Sep. 27, 2000, provisional application No. 60/284,448, filed on Apr. 18, 2001.

(51) **Int. Cl.**
A61K 31/70 (2006.01)
A61K 31/685 (2006.01)
A61K 31/50 (2006.01)
A61K 31/525 (2006.01)
A61K 31/519 (2006.01)

(52) **U.S. Cl.** **514/52**; 514/77; 514/249; 514/251; 514/265.1

(58) **Field of Classification Search** 514/52, 514/77, 249, 251, 265.1
See application file for complete search history.

(56) **References Cited**

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Primary Examiner—Kevin Weddington
(74) *Attorney, Agent, or Firm*—Elizabeth A. McGraw

(57) **ABSTRACT**

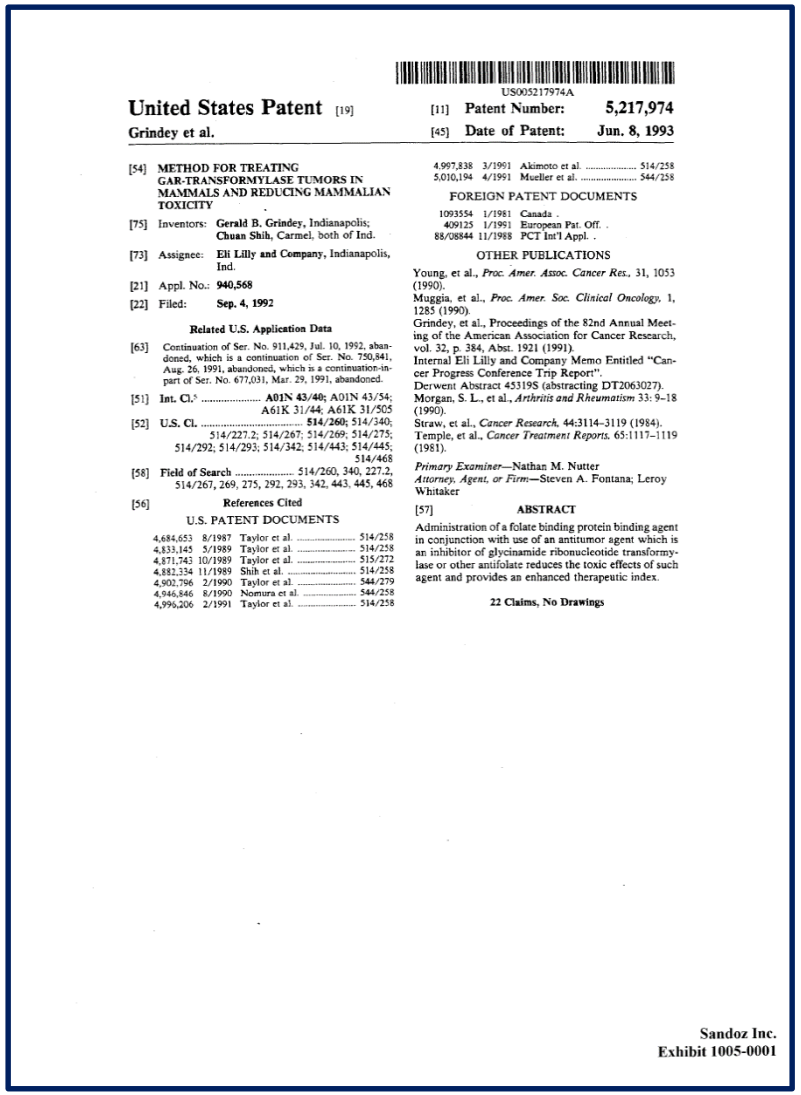
A method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1001-0001

Ex. 1001

Prior Art: ’974 Patent (Ex. 1005)



US005217974A

United States Patent [19]
Grindey et al.

[11] **Patent Number: 5,217,974**
[45] **Date of Patent: Jun. 8, 1993**

[54] **METHOD FOR TREATING GAR-TRANSFORMYLASE TUMORS IN MAMMALS AND REDUCING MAMMALIAN TOXICITY**

[75] Inventors: **Gerald B. Grindey**, Indianapolis; **Chuan Shih**, Carmel, both of Ind.

[73] Assignee: **Eli Lilly and Company**, Indianapolis, Ind.

[21] Appl. No.: **940,568**

[22] Filed: **Sep. 4, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 911,429, Jul. 10, 1992, abandoned, which is a continuation of Ser. No. 750,841, Aug. 26, 1991, abandoned, which is a continuation-in-part of Ser. No. 677,031, Mar. 29, 1991, abandoned.

[51] **Int. Cl.**[5] **A01N 43/40**; A01N 43/54; A61K 31/44; A61K 31/505

[52] **U.S. Cl.** **514/260**; 514/340; 514/227.2; 514/267; 514/269; 514/275; 514/292; 514/293; 514/342; 514/443; 514/445; 514/468

[58] **Field of Search** 514/260, 340, 227.2, 514/267, 269, 275, 292, 293, 342, 443, 445, 468

[56] **References Cited**

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Temple, et al., *Cancer Treatment Reports*, 65:1117–1119 (1981).

Primary Examiner—Nathan M. Nutter
Attorney, Agent, or Firm—Steven A. Fontana; Leroy Whitaker

[57] **ABSTRACT**

Administration of a folate binding protein binding agent in conjunction with use of an antitumor agent which is an inhibitor of glycinamide ribonucleotide transformylase or other antifolate reduces the toxic effects of such agent and provides an enhanced therapeutic index.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1005-0001

The FBP binding agent is administered to the subject mammal prior to treatment with the GAR-transformylase inhibitor or other antifolate. Pretreatment with the suitable amount of FBP binding agent from about 1 to about 24 hours is usually sufficient to substantially bind to and block the folate binding protein prior to administration of the GAR-transformylase inhibitor or other antifolate. Although one single dose of the FBP binding agent, preferably an oral administration of folic acid, should be sufficient to load the folate binding protein, multiple dosing of the FBP binding agent can be employed for periods up to weeks before treatment with the active agent to ensure that the folate binding protein is sufficiently bound in order to maximize the benefit derived from such pretreatment.

In the especially preferred embodiment of this invention, about 1 mg to about 5 mg of folic acid is administered orally to a mammal about 1 to about 24 hours prior to the parenteral administration of the amount of lomotrexol which is normally required to attain the desired therapeutic benefit. Although greater or additional doses of folic acid or another FBP binding agent are also operable, the above parameters will usually bind the folate binding protein in an amount sufficient to reduce the toxicity effects normally seen upon lomotrexol administration above.

Ex. 1005, col. 6, ll. 22-47; Ex. 1004, Schiff Decl., ¶¶112-14; Ex. 1075, Schiff Reply, ¶¶117-21.

Dosing Schedules

Q. Doctor, I want to put us in the pre-June 1999 time frame. Okay? And are you aware of physicians, when they're starting a patient on an antifolate like methotrexate, that they -- they delay the initiation of treatment to accommodate the person's personal schedules? For example, they have a wedding coming up in a few days or they're taking trip. We'll start when you get back in a week?

A. Well, I think it depends on the tumor. If they've got leukemia, I don't think they'd be doing that. If it's something where you don't need an immediate response, you might delay for certain reasons.

Q. Okay. And one of ordinary skill would understand that, in June of 1999, that you could delay the onset of treatment depending on the type of tumor?

A. The circumstances. Yes.

Q. And what kind of delays would we typically see in that type of situation? Are we talking days, weeks, months?

A. Weeks.

Ex. 1074, Chabner Dep. 296:23-297:20.; Ex . 1075, Schiff Reply, ¶121

121. Finally, Dr. Chabner's contention that a POSA would not pretreat for a period longer than 2 days ignores the reality of cancer treatment. As even Dr. Chabner acknowledged, oncologists often will permit patients to postpone the initiation of chemotherapy for several days, or even weeks, in order to accommodate the patient's schedule (e.g., for a wedding or important work commitment). (Ex. 1074, Chabner Dep. 296:23-297:20.) The POSA would simply administer vitamin B_{12} and instruct the patient to begin folic acid pretreatment during the initial appointment in which the patient was consented for pemetrexed chemotherapy.

Ex. 1075, Schiff Reply, ¶ 121.

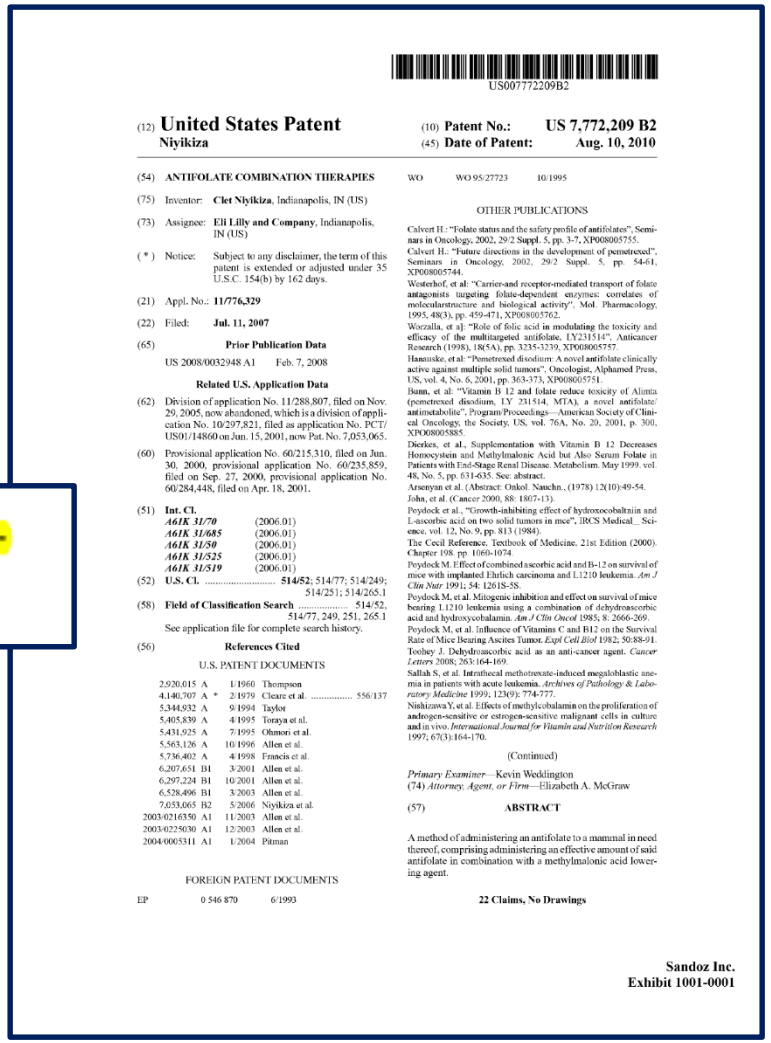
Prior Art Disclosures

COMBINATION WITH CISPLATIN

'209 Patent: Dependent Claims 11, 13, and 22 Also Require Administration of Cisplatin

Claim 22

22. The method of claim 21 further comprising the administration of cisplatin to the patient.



US007772209B2

(12) **United States Patent**
Niyikiza

(10) **Patent No.: US 7,772,209 B2**
(45) **Date of Patent: Aug. 10, 2010**

(54) **ANTIFOLATE COMBINATION THERAPIES**

(75) Inventor: **Clet Niyikiza**, Indianapolis, IN (US)

(73) Assignee: **Eli Lilly and Company**, Indianapolis, IN (US)

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(22) Filed: **Jul. 11, 2007**

(65) **Prior Publication Data**

US 2008/0032948 A1 Feb. 7, 2008

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(60) Provisional application No. 60/215,310, filed on Jun. 30, 2000, provisional application No. 60/235,859, filed on Sep. 27, 2000, provisional application No. 60/284,448, filed on Apr. 18, 2001.

(51) **Int. Cl.**

A61K 31/70	(2006.01)
A61K 31/685	(2006.01)
A61K 31/50	(2006.01)
A61K 31/525	(2006.01)
A61K 31/519	(2006.01)

(52) **U.S. Cl.** **514/52**; 514/77; 514/249; 514/251; 514/265.1

(58) **Field of Classification Search** 514/52, 514/77, 249, 251, 265.1

See application file for complete search history.

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(Continued)

Primary Examiner—Kevin Weddington
(74) *Attorney, Agent, or Firm*—Elizabeth A. McGraw

(57) **ABSTRACT**

A method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1001-0001

Ex. 1001

Prior Art: Thödtmann

Preliminary Results of a Phase I Study With MTA (LY231514) in Combination With Cisplatin in Patients With Solid Tumors

Ralf Thödtmann, Henrik Depenbrock, Johannes Blatter, Robert D. Johnson, Allan van Oosterom, and Axel-R. Hanauske

MTA (multitargeted antifolate, LY231514) is a novel antimetabolite resulting from structure/activity studies of the lometrexol-type antifolates. It has been shown to inhibit various enzymes of folate pathways and has broad antitumor activity in a variety of in vitro and in vivo tumor models. Clinical phase I studies have been performed using different administration schedules, and subsequently the every-21-days schedule has been selected for further development. We report the preliminary findings from a combination phase I study of MTA and cisplatin administered every 21 days. In the first cohort (34 patients), both agents were administered on day 1 with a starting dose of 300 mg/m² MTA and 60 mg/m² cisplatin. In a second cohort (10 patients), MTA (500 or 600 mg/m²) was administered on day 1 followed by cisplatin (75 mg/m²) on day 2. The maximum tolerated doses were reached at 600 mg/m² MTA/100 mg/m² cisplatin (cohort 1) and 600 mg/m² MTA/75 mg/m² cisplatin (cohort 2). In cohort 1, dose-limiting toxicities consisted of reversible myelosuppression with leukopenia and neutropenia. In addition, delayed fatigue also was of clinical significance. Pharmacokinetic analyses indicated that hydration administered before the administration of cisplatin did not influence the major pharmacokinetic parameters of MTA. Eleven objective remissions were observed, including one complete response in a patient with relapsed squamous cell carcinoma of the head and neck and partial responses in four of seven patients with mesothelioma. In contrast, the dose-limiting toxicities in patient cohort 2 consisted of neutropenic sepsis, diarrhea, and skin toxicity with two possibly treatment-related deaths on study. No objective remissions are presently observed in cohort 2. We conclude that the combination of MTA and cisplatin is feasible and clinically active when both agents are administered on day 1 and that it should be pursued for further clinical development.

Semin Oncol 26 (suppl 6):89–93. Copyright © 1999 by W.B. Saunders Company.

MTA (multitargeted antifolate, LY231514, N-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]-benzoyl]-L-glutamic acid) is a novel antimetabolite resulting from structure/activity studies of the lometrexol-type antifolates.[1,2] After cellular uptake, the compound undergoes polyglutamation with production of predominantly triglutamates and pentaglutamates.[3] MTA, as well as its polyglutamates, has been shown to inhibit various enzymes of the folate pathways, including thymidylate synthase, dihydrofolate reductase, glycinamide ribonucleotide formyltransferase, and aminoimidazole carboxamide ribonucleotide formyltransferase.[3] The compound arrests CCRF-CEM cells at the G1/S transition and has been shown to induce apoptosis in these cells.[4] MTA has broad antitumor activity in a variety of in vitro tumor models and is active against lymphoma, colon, lung, pancreas, and breast cancer xenografts in vivo.[5] The preclinical toxicology studies demonstrated that nutritional folate supplementation decreased toxicity of the compound while enhancing its activity.[5]

Clinical phase I studies have been performed using three different administration schedules (every 21 days, daily ×5 every 3 weeks, weekly ×4 every 6 weeks).[6-8] Based on the toxicity profile, the ability to give repeat doses, and the ease of administration, the every-21-days schedule was subsequently selected for further development of MTA in clinical phase II studies. At present, several single-agent phase II studies are in progress or under analysis and MTA appears to be active in the colon, pancreas, and breast cancer. We report here the preliminary results of a phase I combination study of MTA and cisplatin.

PATIENTS AND METHODS

The objectives of the study were to determine the maximum tolerated dose and the dose-limiting toxicity (DLT) of MTA when combined with cisplatin, to recommend a safe and feasible dose and schedule for subsequent phase II studies, and to collect anecdotal information on the antitumor activity of this

From the Universitair Ziekenhuis Gasthuisberg, Katholic University of Leuven, Belgium; the Eli Lilly Company, Bad Homburg, Germany; and Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN.

Sponsored by Eli Lilly and Company.

Dr Thödtmann has received honorarium from Eli Lilly and Company. Drs Blatter and Johnson are employees of Eli Lilly and Company. Dr Hanauske is a consultant for Eli Lilly and Company and Ilex Oncology; has received honoraria from Eli Lilly and Company, Yewtree, Nycomed, and Ilex Oncology; has received research support from Eli Lilly and Company, SmithKline Beecham, Asta Medical, Rhône-Poulenc Rorer, and Essi, and is a provider of expert testimony for Rhône-Poulenc Rorer.

Address reprint requests to Axel-R. Hanauske, MD, PhD, Center for Hematology and Oncology, Landwehrstr 2, D-80336 München, Germany.

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0093-7754/99/2602-0614$10.00/0

Sandoz Inc.
Exhibit 1017-0001

Ex. 1017, Thodtmann

MTA (multitargeted antifolate, LY231514) is a novel antimetabolite resulting from structure/activity studies of the lometrexol-type antifolates. It has been shown to inhibit various enzymes of folate pathways and has broad antitumor activity in a variety of in vitro and in vivo tumor models. Clinical phase I studies have been performed using different administration schedules, and subsequently the every-21-days schedule has been selected for further development. We report the preliminary findings from a combination phase I study of MTA and cisplatin administered every 21 days. In the first cohort (34 patients), both agents were administered on day 1 with a starting dose of 300 mg/m² MTA and 60 mg/m² cisplatin. In a second cohort (10 patients), MTA (500 or 600 mg/m²) was administered on day 1 followed by cisplatin (75 mg/m²) on day 2. The maximum tolerated doses were reached at 600 mg/m² MTA/100 mg/m² cisplatin (cohort 1) and 600 mg/m² MTA/75 mg/m² cisplatin (cohort 2). In cohort 1, dose-limiting toxicities consisted of reversible myelosuppression with leukopenia and neutropenia. In addition, delayed fatigue also was of clinical significance. Pharmacokinetic analyses indicated that hydration administered before the administration of cisplatin did not influence the major pharmacokinetic parameters of MTA. Eleven objective remissions were observed, including one complete response in a patient with relapsed squamous cell carcinoma of the head and neck and partial responses in four of seven patients with mesothelioma. In contrast, the dose-limiting toxicities in patient cohort 2 consisted of neutropenic sepsis, diarrhea, and skin toxicity with two possibly treatment-related deaths on study. No objective remissions are presently observed in cohort 2. We conclude that the combination of MTA and cisplatin is feasible and clinically active when both agents are administered on day 1 and that it should be pursued for further clinical development.

Ex. 1017, Thodtmann at 89.

Secondary Considerations

PURPORTED SKEPTICISM

Dr. Chabner's Purported Skepticism Based on Farber (1948) and "prior experiments" with "poor results"

225. When I first heard that Lilly had decided to supplement patients receiving pemetrexed with vitamin B12 and folic acid, I was skeptical that it would work. I was concerned that the use of such vitamins, and in particular supplementation prior to treatment with the antifolate, would undermine the efficacy of pemetrexed. My skepticism was based on the fact that folates and antifolates have long been known to have a competitive relationship, a relationship which I studied and reported on extensively, and providing vitamin B12 and folic acid prior to treatment would be expected to shift the competitive balance in favor of the folates, and thus would be counterproductive. I was also aware that prior experiments involving folic acid pretreatment with antifolates had generated poor results. In addition, I was concerned that the vitamin supplementation could facilitate the growth of the tumor along the lines of the acceleration phenomenon observed by Dr. Farber.

Ex. 2120, Chabner Decl. ¶2120.

Purported Skepticism: Lometrexol + Folic Acid

Laohavinij (1996)

In summary, the work described in this report has demonstrated that lometrexol toxicity can be modulated by folic acid supplementation in patients. The information obtained from both preclinical murine, and the clinical Phase I study of lometrexol with folate supplementation reported here, indicates that the MTD of lometrexol given with folate supplementation may be higher than the current dose level. The mechanism responsible for the reduction in lometrexol toxicity has not been defined, although associated pharmacokinetic studies suggest that folic acid is not acting by enhancing lometrexol plasma clearance [12]. This work has identified for the first time a safe and acceptable clinical schedule for the administration of a GARFT inhibitor, and the information obtained from this study will facilitate the future development and evaluation of this class of compounds in the treatment of human cancer.

Ex. 2031, Laohavinij at 333-334.

Mendelsohn (1999)

LY309887. As has been reviewed earlier in this chapter, LY309887 has a different ratio of binding the different isoforms of the folate receptor, such that one would predict a greater distribution of the molecule into target tumor compared with the liver, and consequently, an improved therapeutic index. In preclinical models of efficacy, LY309887 appears to be more active than lometrexol in two pancreatic xenografts and the LX1 lung model (Table 4). Therefore, on completion of the preclinical toxicology for the compound, Eli Lilly decided to discontinue development of lometrexol in favor of developing LY309887. Hence, no phase II studies were conducted with lometrexol to assess efficacy.

Ex. 1012, Mendelsohn at 277; Ex. 1075, Schiff Reply, ¶48.

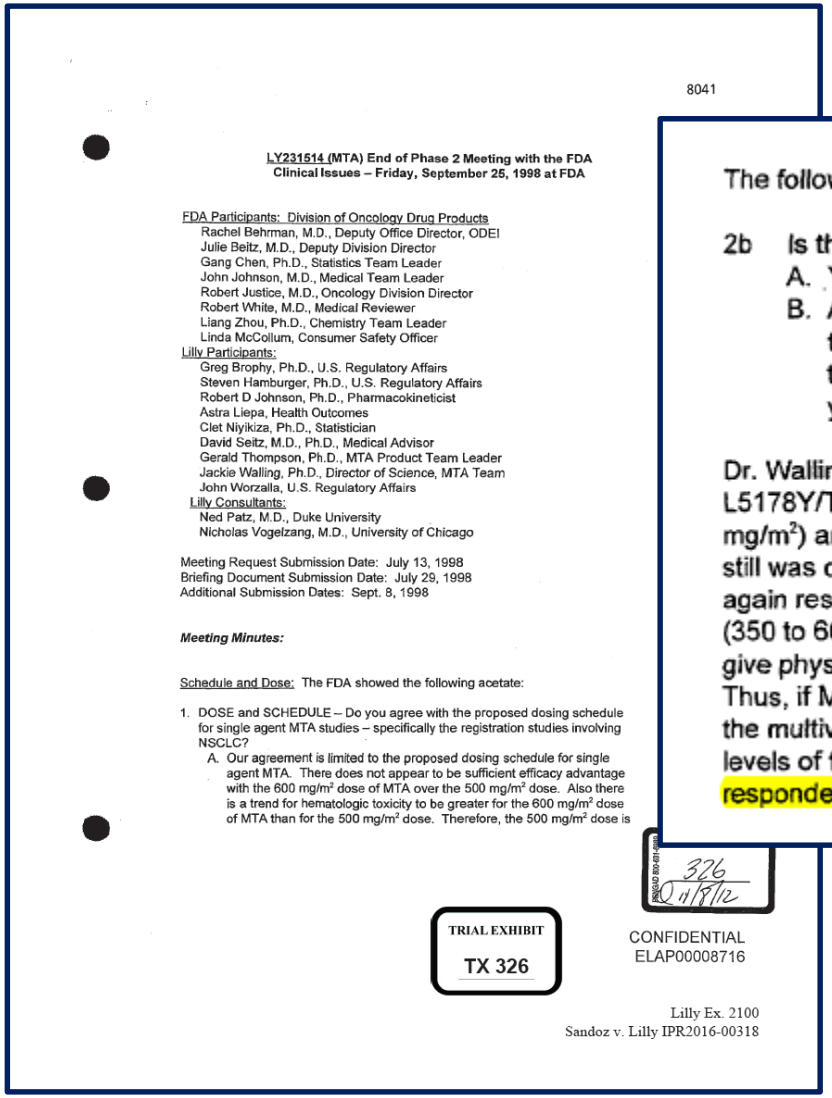
Tularik Clinical Trial (2002)

Lometrexol Plus Folic Acid in Treating Patients With Stage IIIB or Stage IV Non-Small Cell Lung Cancer

RATIONALE: **Lometrexol** may stop or slow the growth of tumor cells by blocking the enzymes necessary for tumor cell growth. Folic acid may be effective in preventing or lessening the side effects of **lometrexol**. Combining **lometrexol** with folic acid may be an effective treatment for non-small cell lung cancer.

Ex. 1099, clinicaltrials.gov; Ex. 1075, Schiff Reply, ¶49.

Purported Skeptism: FDA Statements



8041

LY231514 (MTA) End of Phase 2 Meeting with the FDA
Clinical Issues – Friday, September 25, 1998 at FDA

FDA Participants: Division of Oncology Drug Products
Rachel Behrman, M.D., Deputy Office Director, ODEI
Julie Beitz, M.D., Deputy Division Director
Gang Chen, Ph.D., Statistics Team Leader
John Johnson, M.D., Medical Team Leader
Robert Justice, M.D., Oncology Division Director
Robert White, M.D., Medical Reviewer
Liang Zhou, Ph.D., Chemistry Team Leader
Linda McCollum, Consumer Safety Officer

Lilly Participants:
Greg Brophy, Ph.D., U.S. Regulatory Affairs
Steven Hamburger, Ph.D., U.S. Regulatory Affairs
Robert D Johnson, Ph.D., Pharmacokineticist
Astra Liepa, Health Outcomes
Clet Niyikiza, Ph.D., Statistician
David Seitz, M.D., Ph.D., Medical Advisor
Gerald Thompson, Ph.D., MTA Product Team Leader
Jackie Walling, Ph.D., Director of Science, MTA Team
John Worzalla, U.S. Regulatory Affairs

Lilly Consultants:
Ned Patz, M.D., Duke University
Nicholas Vogelzang, M.D., University of Chicago

Meeting Request Submission Date: July 13, 1998
Briefing Document Submission Date: July 29, 1998
Additional Submission Dates: Sept. 8, 1998

Meeting Minutes:

Schedule and Dose: The FDA showed the following acetate:

1. DOSE and SCHEDULE – Do you agree with the proposed dosing schedule for single agent MTA studies – specifically the registration studies involving NSCLC?
   A. Our agreement is limited to the proposed dosing schedule for single agent MTA. There does not appear to be sufficient efficacy advantage with the 600 mg/m^2 dose of MTA over the 500 mg/m^2 dose. Also there is a trend for hematologic toxicity to be greater for the 600 mg/m^2 dose of MTA than for the 500 mg/m^2 dose. Therefore, the 500 mg/m^2 dose is

TRIAL EXHIBIT
TX 326

326
D 11/8/12

CONFIDENTIAL
ELAP00008716

Lilly Ex. 2100
Sandoz v. Lilly IPR2016-00318

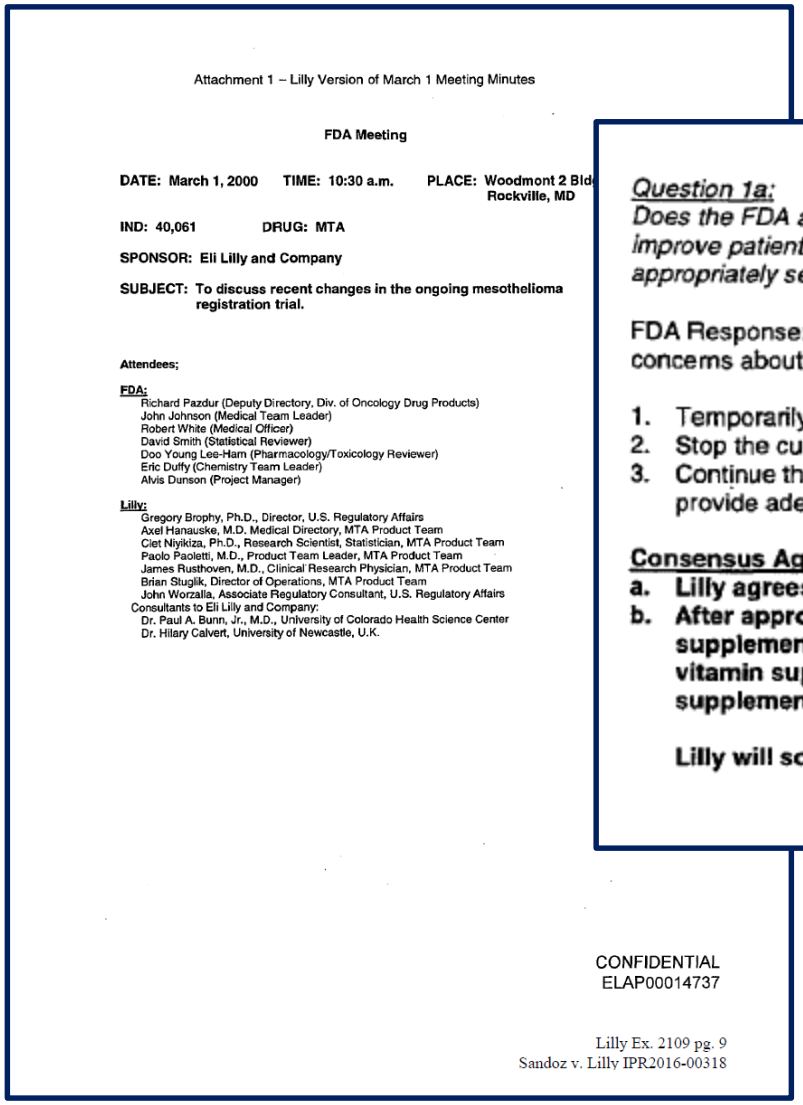
The following FDA acetate for issue 2b was shown:

2b Is the design of study (JMCH) adequate and well controlled?
   A. Yes, with reservations. This would be a better study if it were blinded.
   B. A randomized trial of MTA + cisplatin vs. cisplatin alone is an adequate trial. However the addition of the vitamins to the MTA arm without data that efficacy is not reduced is risky. We would like to know the basis for your determination that the addition of vitamins will not affect efficacy.

Dr. Walling answered this with an acetate (#3) with preclinical data from a murine L5178Y/TK-/Hx- lymphoma tumor model showing that folic acid at 15 mg/kg (45 mg/m^2) ameliorates the toxicity of MTA, but it does not affect the efficacy. There still was concern from the FDA that folic acid might reduce efficacy. Dr. Walling again responded that we are using low doses of folic acid that are in a range (350 to 600 µg/day which is similar to the 100% RDA of 400 µg/day) that would give physiologic levels that might be expected from dietary exposure to folate. Thus, if MTA efficacy was negatively impacted by these low levels of folic acid in the multivitamins, then the activity of MTA would be compromised by similar levels of folate that could be ingested with food in a normal diet. The FDA responded that it was Lilly's decision whether or not to use folate.

Ex. 2100, Sept. 1998 FDA Minutes at 8044; Paper 36, PO Resp. at 58.

Purported Skepticism: FDA Statements



Attachment 1 – Lilly Version of March 1 Meeting Minutes

FDA Meeting

DATE: March 1, 2000 TIME: 10:30 a.m. PLACE: Woodmont 2 Bld Rockville, MD

IND: 40,061 DRUG: MTA

SPONSOR: Eli Lilly and Company

SUBJECT: To discuss recent changes in the ongoing mesothelioma registration trial.

Attendees;

FDA:
Richard Pazdur (Deputy Directory, Div. of Oncology Drug Products)
John Johnson (Medical Team Leader)
Robert White (Medical Officer)
David Smith (Statistical Reviewer)
Doo Young Lee-Ham (Pharmacology/Toxicology Reviewer)
Eric Duffy (Chemistry Team Leader)
Alvis Dunson (Project Manager)

Lilly:
Gregory Brophy, Ph.D., Director, U.S. Regulatory Affairs
Axel Hanauske, M.D. Medical Directory, MTA Product Team
Ciet Niyikiza, Ph.D., Research Scientist, Statistician, MTA Product Team
Paolo Paoletti, M.D., Product Team Leader, MTA Product Team
James Rusthoven, M.D., Clinical Research Physician, MTA Product Team
Brian Stuglik, Director of Operations, MTA Product Team
John Worzalla, Associate Regulatory Consultant, U.S. Regulatory Affairs
Consultants to Eli Lilly and Company:
Dr. Paul A. Bunn, Jr., M.D., University of Colorado Health Science Center
Dr. Hilary Calvert, University of Newcastle, U.K.

CONFIDENTIAL
ELAP00014737

Lilly Ex. 2109 pg. 9
Sandoz v. Lilly IPR2016-00318

Question 1a:
Does the FDA agree that toxicity and mortality data support a programmatic intervention to improve patient safety in LY231514 trials and that daily low dose folic acid supplementation appropriately serves this purpose.

FDA Response: The addition of vitamins to the pivotal trial(s) is at Lilly's risk. We share your concerns about toxicity: your options include:

1. Temporarily closing the trial and conducting a new Phase 1 trial with MTA + vitamins.
2. Stop the current trial and open a new trial using a new protocol and new dose.
3. Continue the current trial with the addition of vitamins and with a recalculated sample size to provide adequate power for comparisons.

Consensus Agreement for Question 1a

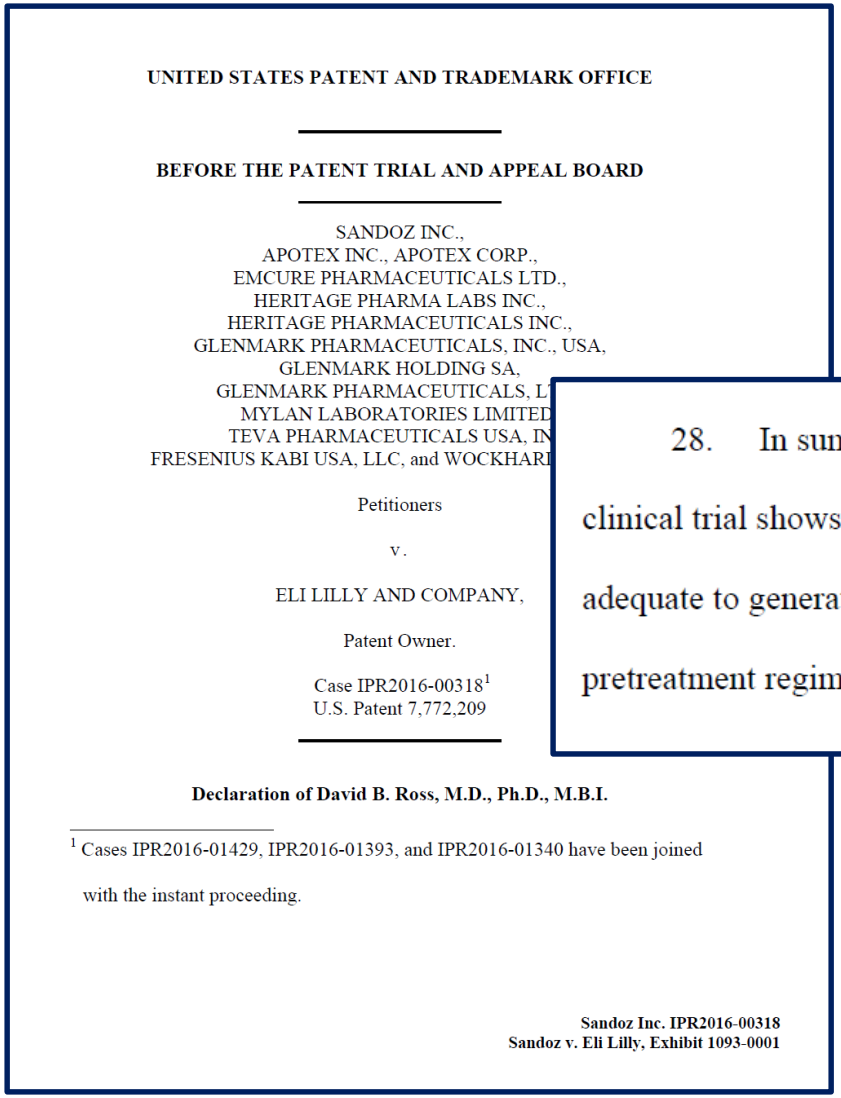
a. Lilly agrees to option #3

b. After approx. 150 patients are treated on the revised protocol with vitamin supplementation, a survival analysis will be done pooling the approx. 150 patients with vitamin supplementation with the approx. 150 patients without vitamin supplementation

Lilly will soon submit to the FDA a prospective detailed plan for the analysis.

Ex. 2109, Mar. 20, 2000 FDA Minutes at 10; Paper 36, PO Resp. at 58; Ex. 2132, Ross Dep. 103:11-24.

Purported Skepticism: FDA Statements



UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SANDOZ INC.,
APOTEX INC., APOTEX CORP.,
EMCURE PHARMACEUTICALS LTD.,
HERITAGE PHARMA LABS INC.,
HERITAGE PHARMACEUTICALS INC.,
GLENMARK PHARMACEUTICALS, INC., USA,
GLENMARK HOLDING SA,
GLENMARK PHARMACEUTICALS, L
MYLAN LABORATORIES LIMITED
TEVA PHARMACEUTICALS USA, IN
FRESENIUS KABI USA, LLC, and WOCKHARI

Petitioners

v.

ELI LILLY AND COMPANY,

Patent Owner.

Case IPR2016-00318[1]
U.S. Patent 7,772,209

Declaration of David B. Ross, M.D., Ph.D., M.B.I.

[1] Cases IPR2016-01429, IPR2016-01393, and IPR2016-01340 have been joined with the instant proceeding.

Sandoz Inc. IPR2016-00318
Sandoz v. Eli Lilly, Exhibit 1093-0001

28. In summary, the fact that the FDA had Lilly add more patients to the clinical trial shows that the FDA was skeptical that the design of the trial was adequate to generate statistically significant results, not whether the vitamin pretreatment regimen would be safe and effective for patients.

Ex. 1093, Ross Decl. ¶28.

Purported Skepticism: FDA Statements

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

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APOTEX INC., APOTEX CORP.,
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HERITAGE PHARMA LABS INC.,
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GLENMARK HOLDING SA,
GLENMARK PHARMACEUTICALS, LTD.,
MYLAN LABORATORIES LIMITED,
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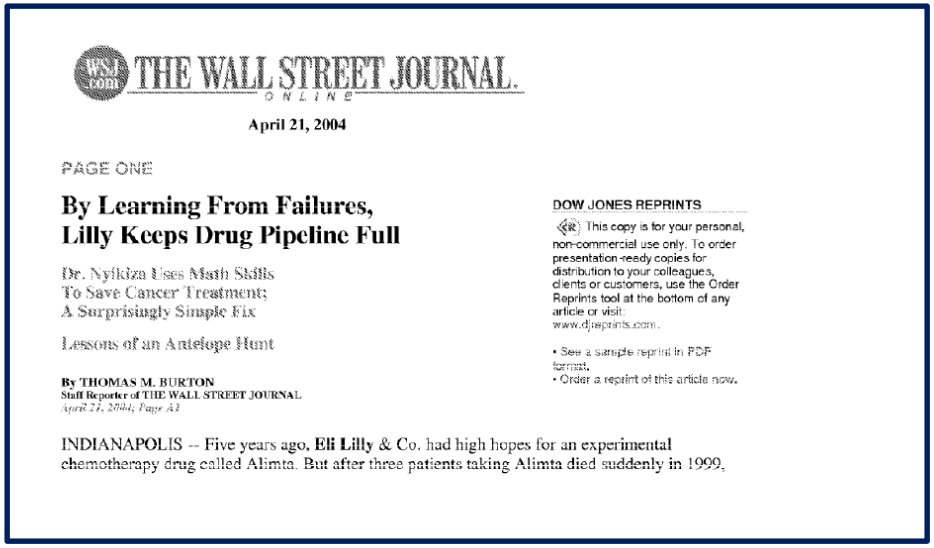
[1] Cases IPR2016-01429, IPR2016-01393, and IPR2016-01340 have been joined with the instant proceeding.

Sandoz Inc. IPR2016-00318
Sandoz v. Eli Lilly, Exhibit 1093-0001

27. Finally, I note that Lilly's reading of "at risk" as reflecting the Agency's judgment about the safety of the vitamin pretreatment regimen is not supported by the changes accepted by the FDA and the FDA's role to ensure patient safety. If the FDA thought that the vitamin pretreatment regimen would expose patients to an unreasonable and significant risk of illness or injury, particularly because the regimen decreased the probability of survival of patients receiving the regimen, then the FDA would have had the authority to suspend the trial by issuing an order known as a "clinical hold." 21 C.F.R. § 312.42(b)(1)(i), (b)(2)(i) (1999). The FDA did just that with pemetrexed: the FDA placed a clinical hold on a pemetrexed trial in 1992 because of the inadequacy of the pre-clinical (animal) data to support the proposed starting dose. (Ex. 1119 at 19.) The FDA will normally attempt to discuss and satisfactorily resolve a potential safety issue with the sponsor before issuing a clinical hold. 21 C.F.R. § 312.42(c) (1999).

Ex. 1093, Ross Decl. ¶28.

Purported Skepticism: Wall Street Journal Article



THE WALL STREET JOURNAL. ONLINE

April 21, 2004

PAGE ONE

By Learning From Failures, Lilly Keeps Drug Pipeline Full

Dr. Nyikiza Uses Math Skills To Save Cancer Treatment; A Surprisingly Simple Fix

Lessons of an Antelope Hunt

By THOMAS M. BURTON
Staff Reporter of THE WALL STREET JOURNAL
April 21, 2004; Page A1

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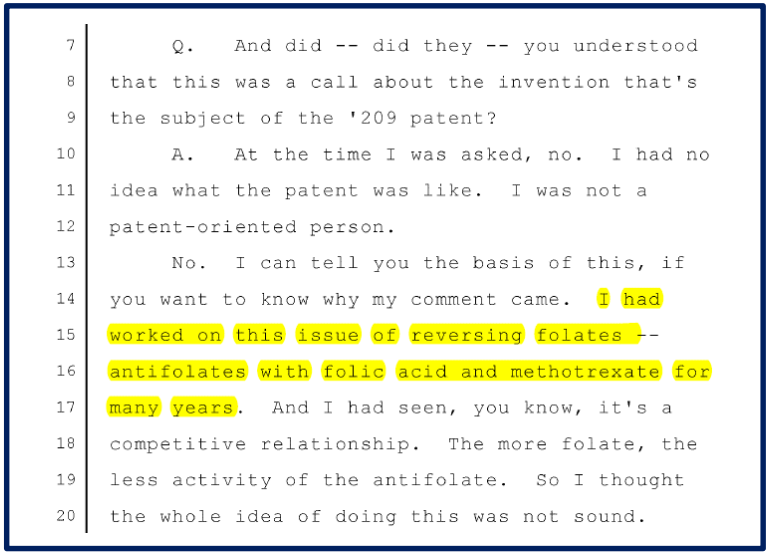
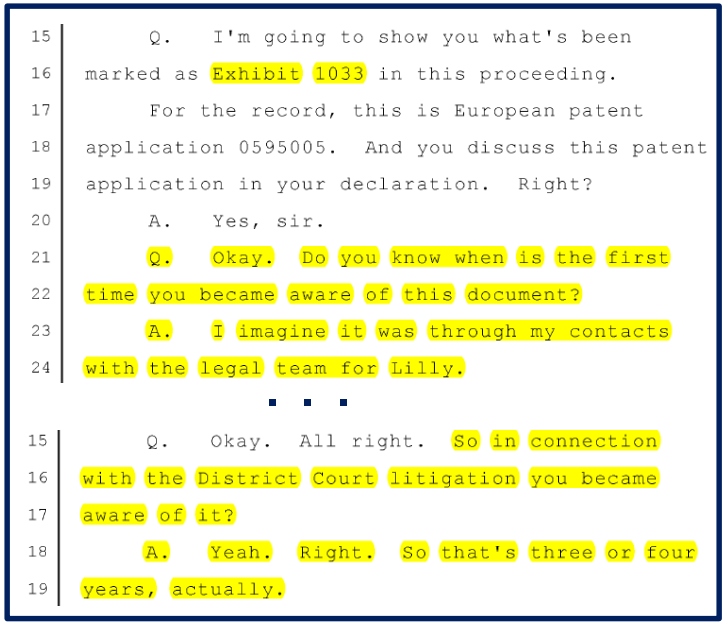
INDIANAPOLIS -- Five years ago, **Eli Lilly** & Co. had high hopes for an experimental chemotherapy drug called Alimta. But after three patients taking Alimta died suddenly in 1999,



Q. And did -- did they -- you understood that this was a call about the invention that's the subject of the '209 patent?

A. At the time I was asked, no. I had no idea what the patent was like. I was not a patent-oriented person.

No. I can tell you the basis of this, if you want to know why my comment came. I had worked on this issue of reversing folates -- antifolates with folic acid and methotrexate for many years. And I had seen, you know, it's a competitive relationship. The more folate, the less activity of the antifolate. So I thought the whole idea of doing this was not sound.



Q. I'm going to show you what's been marked as Exhibit 1033 in this proceeding.

For the record, this is European patent application 0595005. And you discuss this patent application in your declaration. Right?

A. Yes, sir.

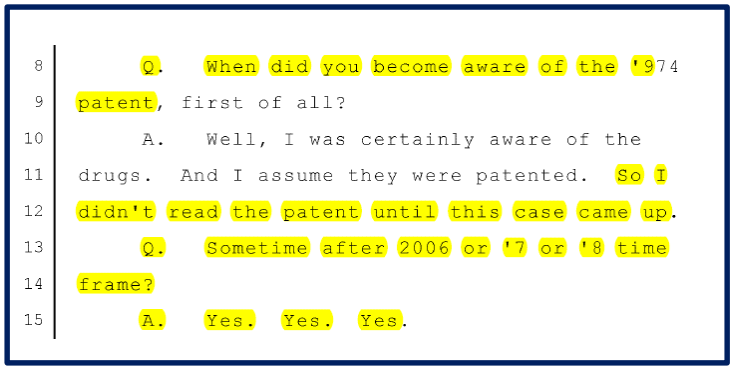
Q. Okay. Do you know when is the first time you became aware of this document?

A. I imagine it was through my contacts with the legal team for Lilly.

. . .

Q. Okay. All right. So in connection with the District Court litigation you became aware of it?

A. Yeah. Right. So that's three or four years, actually.



Q. When did you become aware of the '974 patent, first of all?

A. Well, I was certainly aware of the drugs. And I assume they were patented. So I didn't read the patent until this case came up.

Q. Sometime after 2006 or '7 or '8 time frame?

A. Yes. Yes. Yes.

Paper 49, Sandoz Reply 26-27; Ex. 1074, Chabner Dep. Tr. 182:15-183:14, 282:8-15, 300:7-20

Dr. Chabner's Testimony

Q. Dr. Niyikiza.
A. Yes.
Q. You know him personally?
A. Yes.
Q. Is he a friend of yours?
A. I would say he's an acquaintance.
Q. And not to -- not to peel too many layers away, what do you mean by "acquaintance" versus a "friend"?
A. I mean, I know him.
Q. Okay.
A. He's not one of my personal -- close personal friends.

Q. On Page 192 you were asked the question:
"QUESTION: How do you know Dr. Niyikiza?
"ANSWER: I've known him a long time. I knew him -- when he was at Lilly, I had met him. And then I got to know him very well when he was Glaxo, and now he lives in Boston. He works for Merrimack. He's a very close personal friend. I see him for dinner often and we have a lot of things in common, believe it or not. He's a very, very smart man. He's a wonderful guy."
Did you give that testimony --
A. I did.
Q. -- in April of 2013?
A. Yeah. I used to see him frequently when he was at Merrimack. I haven't since he left. So I see him maybe once or twice a year, since that time. And I wouldn't consider him now a close personal friend.
Q. But you did in 2013?
A. Yeah. I think because he was working in Merrimack and I did see him a lot then when he was there. I also saw him when he was at GSK. And I don't know, you know, whether I knew him at Lilly or not. I mean, I knew of him. I knew who he was, but I don't know if I had met him. Maybe I had met him. But he certainly wasn't a close friend at that time.

Paper 49, Sandoz Reply 22-23; Ex. 1074, 146:6-15, 300:21-303:8

Dr. Chabner's Testimony

Q. Okay. What I'm trying to understand, Doctor, is it says here "Dr. Chabner has consulted for or served on."

So Lilly is the consulted part of this?

A. That's right.

Q. Thank you.

A. It's not an advisory board in the sense that I was telling them how to develop a drug.

Q. Aside from this lawsuit and is the drug you mentioned, any other relationship with Lilly over the years?

A. I have friends that work there.

Q. You, personally?

A. I grew up very close to Indianapolis. Always appreciated the presence of Lilly as an employer in our area.

Q. You like Lilly, I take it?

A. Yes. You know, I have no special like for Lilly. I like companies that actually do something useful for cancer patients. Not all of them do. And Lilly has.

Q. Have you or any of the organizations that you work with received honorariums, financial honorariums from Lilly over the years?

A. You know, I might have at one time. 1996, I think it was, I was asked to be the visiting professor at the Indiana University. And it was called the Eli Lilly Lectureship. I actually showed a picture there of my two dogs in bed with me. And their names were Eli and Lilly. And I said, you know, I've been accused of being in bed with Eli Lilly. It was a joke. And I am. And that is the closest I've been to being in bed with Eli Lilly, yes. They've tended to work with the Dana Farber more than Mass. General.

Q. So the -- so we have this professor chairship, I don't know if that's the right term. But anything else besides this Indiana U?

A. You know, I don't remember anything else. There could have been some other thing, but nothing that I can recall. I mean, if you -- maybe you could refresh my memory, but...

Q. Just curious, why did you name your dogs Eli and Lilly?

A. Because I went to Yale. And then what do you do when you have Eli? You've got to find a woman's name, so it was Lilly.

Paper 49, Sandoz Reply 22-23; Ex. 1074, 67:10-17, 309:20-310:20; *see also id.* at 62:5-64:1

Motion to Exclude: Dr. Niyikiza's Hearsay Testimony

- 9/30/16: PO Resp. filed
- 10/10/16: Sandoz requests deposition of Dr. Niyikiza
- 11/9/16: Sandoz files motion for deposition of Dr. Niyikiza (Paper 43), which was not ruled on
- 2/14/17: Sandoz files motion to exclude (Papers 64, 77)

> Here, the record includes an unusually rich body of contemporaneous reaction to—and skepticism of—the invention that demonstrates that the claimed invention would not have been obvious. As Dr. Niyikiza testified in the Teva Litigation, his idea was met with consistent skepticism, and was not adopted until after the priority date, when deaths occurred in the Phase III clinical trials. Ex. 2116 at 750-58, 760-65, 771-75.[9]

Paper 36, PO Resp. at 57 (citing Ex. 2116, Niyikiza Trial Tr. (Lilly v. Teva))

Motion to Exclude: Dr. Niyikiza's Hearsay Testimony

- Exemplary hearsay statement

> But, also, he came to me and he basically said, "Thanks for what you did. If you didn't do it, this drug would probably be dead."

Ex. 2116, Niyikiza Trial Tr. (Lilly v. Teva) at 845
cited by (Paper 36, PO Resp. at 59)

- Exemplary district court reasoning for allowing such hearsay

> MR. WIESEN: Objection, Your Honor. I think this is plainly going to be hearsay.
>
> MR. GENDERSON: Your Honor, it goes to his state of mind, because it's relevant to what he did later and why he did it.
>
> THE COURT: I'll overrule it. It's interesting. I would like to hear it.

Ex. 2116, Niyikiza Trial Tr. (Lilly v. Teva), 722:25-723:6.

Motion to Exclude: Dr. Zeisel's Testimony

- Agreed definition of POSA:

> 23. In my opinion, the POSA to whom the '209 patent is addressed is a medical doctor who specializes in oncology, specifically medical oncology. Such a person would have knowledge and experience concerning the use of chemotherapy agents, including antifolates, in the treatment of cancer, as well as knowledge and experience regarding the management of toxicities associated with such treatment.

Ex. 2120, Chabner Decl. ¶23; Paper 64 at 12

- Dr. Zeisel's background:

> A Again, I'm not an oncologist, but -- and I would defer to Dr. Chabner.

Ex. 1086, Zeisel Dep. 118:15, 128:19; *see also* 61:6-7.

- Dr. Zeisel's opinions:

> In 1999, the POSA would have been concerned that increasing the folate . . .
>
> As such, the POSA would have understood that the effect of administering . . .

Ex. 2118, Zeisel Decl. ¶¶46, 53

Prior Art Disclosures

NUTRITION + ONCOLOGY

History: Nutrition + Antifolates

Q. Okay. From -- from your standpoint as -- as a biochemist and an expert in folate metabolism, how does pemetrexed work to treat cancer?

A. So pemetrexed is known as a multitargeted antifolate. It's -- there is a long generation of these antifolates. Early in the 1940s, when Lederle Labs first elucidated the structure of folate and synthesized folic acid, almost immediately thereafter they began to design inhibitors or analogs that would inhibit DNA synthesis, because they knew that this compound that they had isolated from biological material was needed for DNA synthesis. Almost immediately they came out with compounds to try to inhibit DNA synthesis.

Ex. 2137, Stover Dep. Tr. 11:17-12:6.

Nutrition + Antifolates: Lilly Statements

Lilly's 2000 Letter to FDA

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Morgan SL, Baggott JE, Lee JY et al. 1998. Folic acid supplementation prevents deficient blood folate levels and hyperhomocysteinemia during longterm, low dose methotrexate therapy for rheumatoid arthritis: Implications for cardiovascular disease prevention. J Rheumatol 25(3):441-446.

Ex. 1076, Feb. 2000 Letter Lilly to FDA at ELAP00013782.

'209 Patent

U.S. Pat. No. 5,217,974. Folic acid has been shown to lower homocysteine levels (see e.g. Homocysteine Lowering Trialist's Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomized trials. BMJ 1998; 316:894-898 and Naurath H J, Joosten E, Riezler R, Stabler S P, Allen R H, Lindenbaum J. Effects of vitamin B 12, folate and vitamin B6 supplements in elderly people with normal serum vitamin concentrations. Lancet 1995; 346:85-89), and homocysteine levels have been shown to be a predictor of cytotoxic events related to the use of GARFT inhibitors, see e.g. U.S. Pat. No. 5,217,974. However, even with this

Ex. 1001, '209 Patent, col. 2, ll. 20-24; Paper 42, Sandoz Reply at 14-15.

Nutrition + Oncology: Dr. Stover's Testimony

Q. Okay. And, but my question is: Have you -- have you made any effort in your mind to separate out, you know, what you know and what people with your type of expertise would have known from the standpoint of an expert in one-carbon metabolism and biochemistry from what the person of ordinary skill in the art for purposes of this case would have known?

A. I don't see those as distinct, because I can tell you that many of the leading antifolate oncologists attend the same meetings I do. We speak in the same sessions. The focus of the talks is different, but there is a free sharing of information among those groups.

Ex. 2137, Stover Dep. Tr. 26:9-22; .

You're not speaking to what a hypothetical oncologist would or would not have done as of 1999, are you?

A. What I am speaking to is that anyone who was in a hospital setting for treatment would have be -- would have a full clinical team that would include an oncologist. Among that clinical team would be nutrition support, and the clinical oncologist would have that accessible to him or her, and that clinical team, which would hopefully include someone understanding nutrition, if not the oncologist him or herself, would have this information readily available to them, because they come from authoritative sources related to -- to nutrition care.

Ex. 2137, Stover Dep. 109:5-19; Paper 71, Resp. Observation at 3-5.

A. I can tell you as someone who is involved in nutrition, someone who runs one of the largest nutrition programs in the country, that nutrition is the due care about maintaining adequate nutrition in both healthy and diseased populations, that we train nutrition practitioners, registered dieticians in medical nutrition therapy, and that nutrition is an important part of cancer treatment.

Ex. 2137, Stover Dep. Tr. 23:20-24:7.

A. Again, I don't want to speak to what -- I am not a clinician, so I don't want to speak as a clinician.

What I can -- can tell you that I am very active in the scientific community around one-carbon metabolism, folate and B12. At our scientific meetings, which are held annually, we have a blend of people in nutrition, people who are biochemists and clinicians. And so, these discussions occur in that context, an intermingling of the sort of translational spectrum of scientists.

Ex. 2137, Stover Dep. Tr. 22:4-14.

Q. If the oncologist thought to go to an expert in biochemistry and inquire or thought to go to a library that covered, you know, biochemical works --

A. Or nutrition, and nutrition is an important component of clinical care and has been.

Ex. 2137, Stover Dep. Tr. 25:21:26:2.

Dr. Schiff's Testimony Regarding Overlap Between Nutrition and Oncology

Q. And then you say, "and additional qualifications or experience in the field of nutritional sciences involving vitamin deficiencies."

Why did you choose to include that second part in your definition?

A. Because I think that when we talk about things like folic acid metabolism and we talk about known or suspected deficiencies of vitamin B12 or folic acid, that has to be understood for the purpose of administering antifolates safely and effectively.

But there is another reason for that. Most people, especially in private practice, who practice medical oncology also practice hematology. I'm board certified in both of those things, hematology and medical oncology. That means I was evaluating patients with anemia for which vitamin B12 and/or folic acid deficiency was always in the differential diagnosis. And I was referred patients with venous thromboembolism, which meant I had to know something about the role of high homocysteine levels as a risk factor for that. And in the pathophysiology of venous thromboembolism, that involved vitamin B12 and folate metabolism as well.

But there's more than that. Cancer patients often have nutritional compromise based on their disease or on their treatment, and every medical oncologist has to be familiar with how that develops, how to evaluate it and how to manage it.

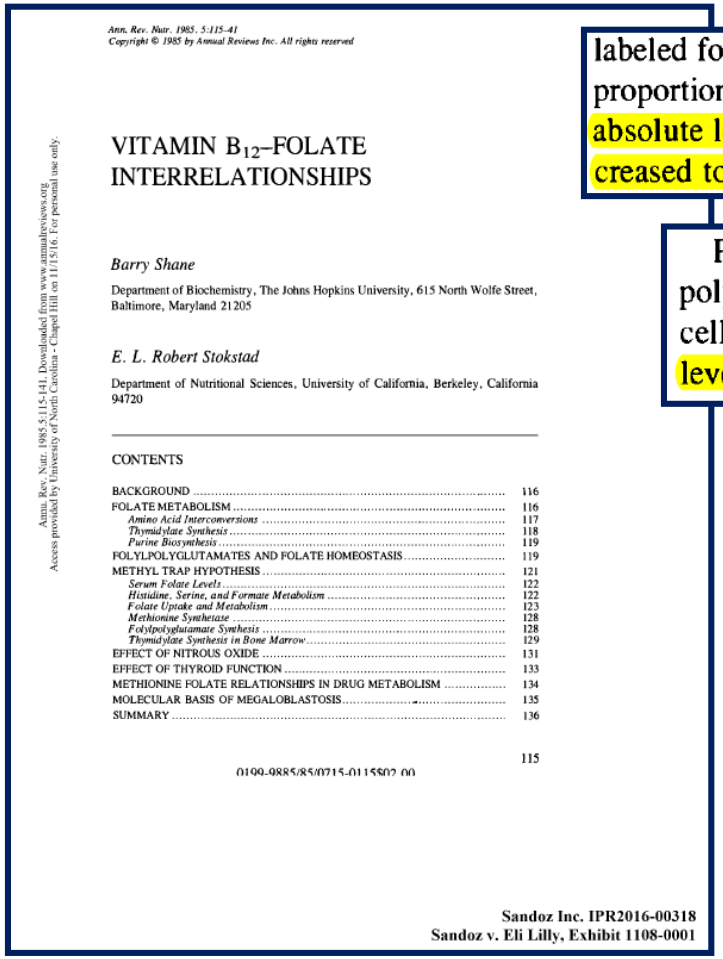
So nutrition is extremely important in medical oncology as well as for the -- the other specific diagnostic and therapeutic applications that I mentioned.

Ex. 2126, Schiff Dep. 43:12-44:22 .

Lilly's Arguments

METHYL TRAP + FOLIC ACID AS AN "ANTIDOTE" TO ANTIFOLATES

Methyl Trap: Results in decreased total tissue folate levels



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VITAMIN B_{12}–FOLATE INTERRELATIONSHIPS

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Sandoz Inc. IPR2016-00318
Sandoz v. Eli Lilly, Exhibit 1108-0001

labeled folate dose. Although vitamin B_{12} deficiency invariably increases the proportion of 5-methyl-$H_4PteGlu_n$ in the livers of experimental animals, the absolute level of methylated folate is often decreased as a result of the decreased total tissue folate levels in these animals.

Practically all the folate derivatives in tissues and blood cells are pteroylpolyglutamates. In vitamin B_{12}/methionine-deficient animals and in red blood cells from pernicious anemia patients, the large drop in endogeneous folate levels is due to a large decrease in folylpolyglutamates (76, 108). The small

Ex. 1108, Shane at 122, 124; Ex. 1091, Stover Reply ¶¶29-30

29. Indeed, the methyl trap was first observed as increased proportions of folate in the 5-mTHF form *and* elevated serum folate levels as 5-mTHF. (Ex. 1108, Shane at 122.) It has been long-known that non-sequestered serum folate is eliminated relatively quickly (Ex. 1114, Krumdieck at abstract.) Thus, while the proportional levels of 5-mTHF are increased during the methyl trap, the total levels of reduced folate decreases because the unpolyglutamated 5-mTHF in the serum is eliminated by the body. Shane also explains that, "Although vitamin B_{12} deficiency invariably increases the proportion of 5-methyl-$H_4PteGlu_n$ [5-mTHF] in the livers of experimental animals, the absolute level of methylated folate is often decreased as a result of the decreased total tissue folate levels in these animals." (Ex. 1108, Shane at 124.) Thus, even if the administration of vitamin B_{12} were to release the trapped 5-mTHF, the resulting folate would not be an overwhelming amount that would push overall folate levels abnormally high and could possibly cause concern about unanticipated effects, such as impeding antifolate efficacy.

Ex. 1091, Stover Reply ¶¶29-30

Methyl Trap: Drs. Schiff and Stover's Testimony

in persons who are suffering from a B_{12} deficiency. Because of the potentially severe neurological effects of an undetected B_{12} deficiency, it has long been engrained in physicians – including oncologists – that steps should be taken to avoid potential vitamin B_{12} deficiencies in the first place. Thus, the possibility of a methyl trap scenario would not be viewed as a serious concern. Indeed, I am unaware of any literature pre-June 1999 expressing any concern about the impact of the methyl trap in cancer patients. Drs. Chabner's and Zeisel's sparse

Ex. 1075, Schiff Reply, ¶99

So we can quantify, and we do know and can estimate with a high degree of certainty, how much folate would be liberated by B12 administration in a methyl trap. And as I stated earlier, I believe in my testimony, that it would elevate that -- that it would make -- the amount of folate that it would make available or release from that trap would be on the order of 20 to 40 percent of total cellular folate, on a background again of pre-existing folate deficiency. So this is -- is a minimal, and I would almost say biochemically insignificant, amount of folate.

Ex. 2137, Stover Dep. 215:4-217:20; Ex. 1091, Stover Reply ¶¶ 29-30; Ex. 1108, Shane, 122, 124; *see also* Paper 68, Patent Owner's Sur-reply at 6-7

Q. But you're sort of a couple steps down from my question. You're talking about how you would cure the methyl trap, right?

A. To the extent it exists. And I mean again, this is something in all my years in practice, I never encountered. And when I first encountered it in preparing for this, I tried to look at the situations and how practically relevant they were.

Ex. 2126, Schiff Dep. 83:6-14; *see also* Paper 68, Sur-reply at 6-7

Lilly's Antidote Argument: Dr. Stover's Testimony Regarding Differences in Folate Catabolism

Lilly's Argument:

> Petitioners cannot (and do not) argue that vitamin B_{12} pretreatment would be expected to serve any useful purpose without creating THF and thus making more folate available to cancer cells as well as healthy ones. Accordingly, if Petitioners

Paper 68, Patent Owner's Sur-reply at 7

> okay, let me put it this way: If you had a tumor that was rapidly catabolizing folate, like a tumor cell does, versus a normal cell, the cell that is undergoing rapid folate catabolism does not respond nearly as well, nor can it achieve the folate levels at a given exposure than a normal cell can, because it's churning the folate, degrading the folate.

Ex. 2137, Stover Dep. 49:16-50:9 *see also* Ex. 1091, Stover Reply ¶ 22; Ex. 1112, Kelly, 305; Ex. 1113, Meenan, 1165, 1167

Lilly's Antidote Argument: Dr. Stover's Testimony Regarding Differences in Folate Catabolism

22. Second, Dr. Zeisel's reliance on the fact that pemetrexed's effects on both cancer and healthy cells work by the "same mechanisms" fails to take into account differences in the cells themselves. It was known in June 1999 that cancer cells waste folate through mechanisms of folate catabolism. Conversely, it was known that healthy cells to not have a high rate of catabolism. For example, tumor bearing mice have been shown to have a mere 5% increase in the weight of the tumour and a 50% increase in folate catabolism (Ex. 1112, Kelly at 305.) Consistent with these experimental findings in animals, Meenan et al. studied the difference of folate in tumor colon epithelial cells and adjacent normal cells and found that "[a] significant difference has been identified between the folate content of colon tumor epithelial cells and that of adjacent normal cells." (Ex. 1113, Meenan at 1165.) This depletion is not simply due to the fact that the cancer cells divide rapidly, but rather is likely due to "abnormal rates of cell turnover or abnormal cellular enzyme activity, such as methylene tetrahydrofolate reductase." (*Id.* pg. 1166.) It makes sense that folic acid supplementation would have a greater impact on protecting healthy cells, which more efficiently use folate, than cancer cells, which catabolize a much higher proportion of the folate received. Notably, Dr. Zeisel acknowledged that he had considered folate metabolism only generally, not specifically catabolism, in forming his opinions. (Ex. 1086, Zeisel Dep. 156:15-157:5.)

Ex. 1091, Stover Reply ¶ 22

Lilly's Antidote Argument: Dr. Stover's Testimony Regarding Differences in Folate Catabolism

GASTROENTEROLOGY 1997;112:1163–1168

Epithelial Cell Folate Depletion Occurs in Neoplastic But Not Adjacent Normal Colon Mucosa

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*Department of Clinical Medicine, Trinity College and St. James's Hospital, Dublin; and ‡Department of Biochemistry, Trinity College, Dublin, Ireland

Background & Aims: **Restricted folate supply is associated with the development of carcinoma, and folate supplements have a protective effect in colorectal carcinoma. This effect may be mediated through correction of local folate deficiency. The aim of this study was to define the folate content of neoplastic colonic epithelial cells and its relation to that of adjacent normal tissue and circulating levels.** ***Methods:*** **Epithelial cells were isolated from endoscopic biopsy specimens of normal, adenocarcinoma, adenoma, and adjacent normal colonic mucosa by ion chelation. Intracellular folate levels were determined by microbiological assay.** ***Results:*** **Folate levels in carcinoma specimens were lower than in adjacent normal tissue ($P < 0.02$). Levels in adenoma epithelial cells were lower than in adjacent normal tissue, although this did not reach statistical significance ($P < 0.06$). Epithelial cells from normal tissue and mucosa adjacent to tumors and adenomata had similar folate contents. Blood folate and vitamin B_{12} indices for all groups were normal.** ***Conclusions:*** **Malignant colon epithelial cells show a relative localized folate deficiency. However, there is no evidence for the occurrence of generalized mucosal folate deficiency. This finding suggests that folate supplements do not inhibit carcinogenesis through correction of localized folate depletion.**

The epidemiology of colorectal carcinoma (CRC) suggests exogenous (dietary constituents) and endogenous (bile acid metabolites) substances to influence mucosal epithelial cell proliferation and progression to malignancy. A protective role has been ascribed to vitamins and micronutrients in carcinogenesis.[1,2] Folate is of particular interest because this vitamin is required for all cellular one-carbon transfer reactions including DNA methylation and thymidine synthesis.[3] Folate supplementation favorably influences epithelial dysplasia in humans,[4,5] reduces colon tumor load in animals exposed to carcinogens,[6] and protects against the development of colonic neoplasia in patients using sulfonamide-containing drugs.[7]

The efficacy of folate in suppressing epithelial neoplasia may be related to the importance of DNA methylation in cell homeostasis. In vitro studies show that focal loss of methyl groups, which influences gene expression and generalized genomic hypomethylation, is a feature of several carcinomas.[8] Increased levels of mucosal genomic methylation after supplementation with supraphysiological doses of folate supports this hypothesis.[9] These and similar studies suggest a general theory that carcinogenesis is related to an epigenetic factor, altered patterns of genomic methylation,[10] although, they do not show a direct link between localized folate depletion and neoplasia.

Low folate levels occur in mixed cell homogenates of potentially premalignant colonic adenomatous polyps.[11] However, folate deficiency has not been shown in cells that produce CRC, the colonic epithelial cell (colonocytes). Furthermore, the relation between the folate content of colon adenoma or tumor and adjacent normal epithelial cells remains to be defined. Consequently, there is little evidence to show that folate supplementation influences carcinogenesis through the correction of tissue folate deficiency.

The aim of this study was to define the folate content of epithelial cells isolated from premalignant and malignant distal colonic lesions and to compare folate content in both adjacent normal colonocytes and tissue specimens taken from nontumor-bearing mucosa.

Materials and Methods

Tissue Collection

Colonic biopsy specimens were obtained at endoscopy under a protocol approved by the Ethics Committee of the Federated Voluntary Dublin Hospitals.

Patient details including medical history and current medication were recorded before endoscopy. Venous blood was drawn for the estimation of red cell folate, serum folate, and vitamin B_{12} levels. Pathology in enrolled patients included distal colonic carcinoma (within 30 cm of the anal verge) (n

Abbreviation used in this report: CRC, colorectal carcinoma.

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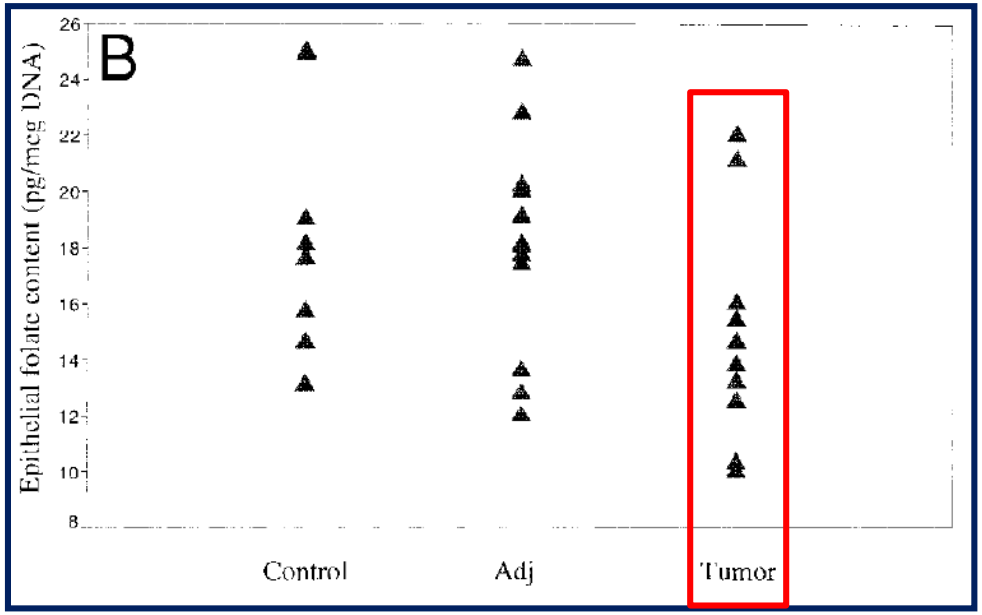
0016-5085/97/$3.00

A significant difference has been identified between the folate content of colon tumor epithelial cells and that of adjacent normal cells. Because enriched epi-



Ex. 1113, Meenan, 1165, 1167; Ex. 1091, Stover Reply ¶ 22

Lilly's Antidote Argument: Dr. Stover's Testimony Regarding Binding Affinities

20. In the cell, folic acid is converted to other forms of folate, including 5,10-methylenetetrahydrofolate. Had Dr. Zeisel considered the binding affinity of pemetrexed for thymidylate synthase (TS), its primary target, versus 5,10-methylenetetrahydofolate, its natural substrate, it would have been apparent that it was known in June of 1999 that folic acid is not an "antidote" for pemetrexed. (Ex. 2118, Zeisel Decl. ¶¶ 25, 50.) The measure of binding affinity for an antifolate like pemetrexed is called the inhibition constant (K_i). The relevant value for the natural substrate (here, 5,10 methylenetetrahydrofolate, which is a reduced form of folic acid), is called the Michaelis-Menten constant (K_m), which is the concentration of substrate (5,10-methylenetetrahydrofolate) required to achieve half maximal velocity of the enzyme. For each constant, lower values indicate less of the substance is required to inhibit (K_i) or activate (K_m) the target enzyme (here, TS). It was known in June 1999 that pemetrexed undergoes a process known as polyglutamation inside the body to become pentaglutamated pemetrexed, and that in this form, pemetrexed was an extremely strong inhibitor of TS, with a K_i of 1.3 ± 0.3 nM. (Ex. 1021, Shih at abstract and 1118.) By contrast, it was known in June 1999, that the form of reduced folic acid that naturally binds to TS was 5,10 methylenetetrahydrofolate, which has a much higher K_m of 3.0 µm. (Ex. 1021, Shih at 1118.) Thus, the relative affinity of the natural substrate (5,10 methylenetetrahydrofolate) to the inhibitor (pemetrexed) was approximately 2,300 (3,000 nM/1.3 nM). This means that approximately 2,300 times more 5,10 methylenetetrahydrofolate is required to activate TS than is required for pemetrexed to inhibit TS. Given this knowledge, pemetrexed would have been expected to outcompete folic acid (in reduced form) for its primary target and thus low levels of folic acid supplementation would not work as an antidote.

Ex. 1091, Stover Reply ¶ 20

Claim Construction

“PATIENT”

Claim Construction of "Patient": Overview of Proposed Constructions

Term	Sandoz's Construction	Lilly's Construction
Patient	"mammal," i.e., all mammals, not limited to humans	"human undergoing medical treatment"

Paper 2, Petition 18-19.

Claim Construction of "Patient": Claims

6. The method of claim **5** wherein the folic acid is administered 1 to 3 weeks prior to the first administration of the pemetrexed disodium.

7. The method of claim **5** wherein the folic acid is administered from about 1 to about 24 hours prior to administration of the pemetrexed disodium.

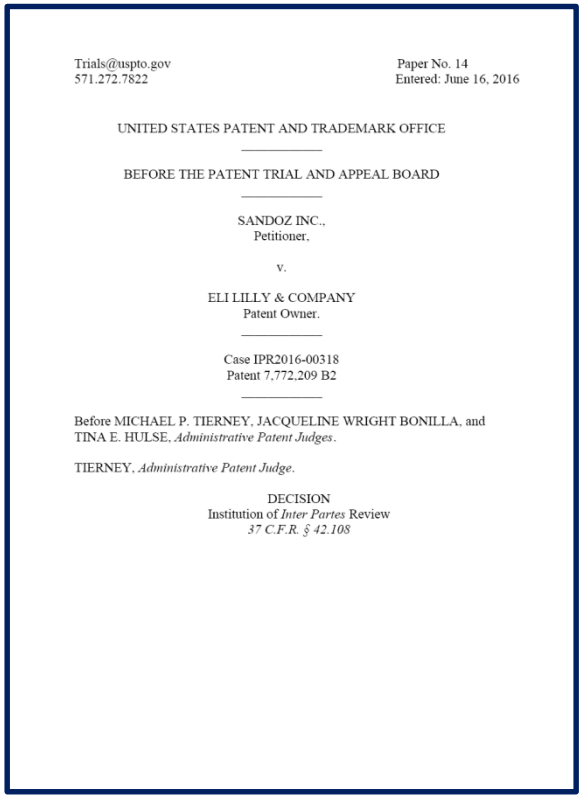
8. The method according to any one of claims **1**-**4**, wherein between 0.3 mg to about 5 mg of folic acid is administered orally.

In the especially preferred embodiment of this invention, about 0.1 mg to about 30 mg, most preferably about 0.3 mg to about 5 mg, of folic acid is administered orally to a mammal about 1 to 3 weeks post administration of the methylmalonic acid lowering agent and about 1 to about 24 hours prior to the parenteral administration of the amount of an antifolate. However, it will be understood that the amount of the methylmalonic acid lowering agent actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual agent administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect.

Compare Ex. 1001, '209 patent, col. 6, ll. 35-49 *with* Ex. 1, claims 6-8; Ex. 1004, Schiff Decl. ¶¶19-22.

Claim Construction of "Patient": Institution

For the reasons provided in detail below, we determine that it is unnecessary to construe explicitly the claim terms for purposes of this Decision. *See Wellman, Inc. v. Eastman Chem. Co.*, 642 F.3d 1355, 1361 (Fed. Cir. 2011) ("[C]laim terms need only be construed 'to the extent necessary to resolve the controversy.'") (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999)).



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Paper No. 14
Entered: June 16, 2016

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SANDOZ INC.,
Petitioner,

v.

ELI LILLY & COMPANY
Patent Owner.

Case IPR2016-00318
Patent 7,772,209 B2

Before MICHAEL P. TIERNEY, JACQUELINE WRIGHT BONILLA, and TINA E. HULSE, *Administrative Patent Judges*.

TIERNEY, *Administrative Patent Judge*.

DECISION
Institution of *Inter Partes* Review
37 C.F.R. § 42.108

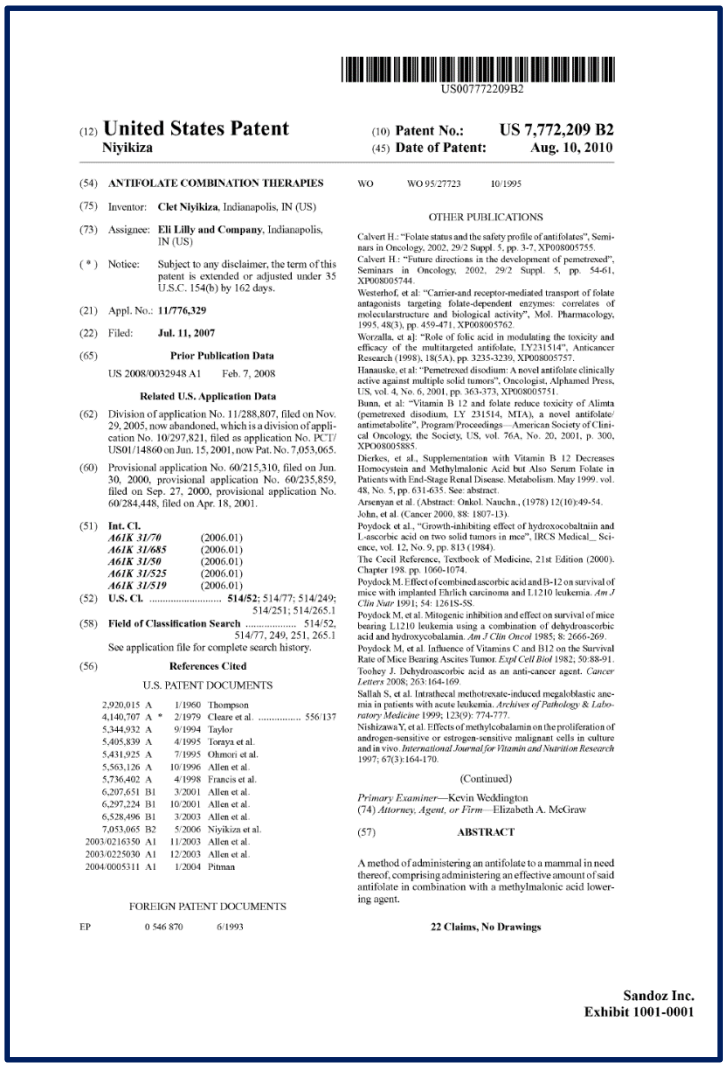
Patent Owner chose not to address the merits of the Petition in its Preliminary Response other than contend that Worzalla's teachings are directed to mice whereas the claims are directed to treating humans. Prelim. Resp. 10, 1 n.1 and 20–29. Additionally, Patent Owner requests that, should the Board institute as to Hammond I, the Board should exercise its discretion and deny institution as to Worzalla as the grounds are redundant. *Id.* at 29–32.

We have considered Patent Owner's contention that Worzalla's teachings are limited to mice but do not find them persuasive at this time. We understand Dr. Schiff as testifying that one skilled in the art would have understood Worzalla's teachings as having applicability to humans and that pretreatment of patients, including humans, would lead to reduced MTA toxicity. Ex. 1004 ¶¶ 24, 53, 65. We credit Dr. Schiff's testimony as Worzalla does not suggest that its teachings, which encompass in vitro treatment of human tumor cell lines and discuss antitumor activity in phase I and II clinical trials, are limited to treatment of cancer in mice. Ex. 1013.

Sandoz DX -147

Paper 14, Institution Decision, 9, 17-18.

Claim Construction of "Patient": Specification



US007772209B2

(12) **United States Patent**
Niyikiza

(10) **Patent No.: US 7,772,209 B2**
(45) **Date of Patent: Aug. 10, 2010**

(54) **ANTIFOLATE COMBINATION THERAPIES**

(75) Inventor: **Clet Niyikiza**, Indianapolis, IN (US)

(73) Assignee: **Eli Lilly and Company**, Indianapolis, IN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 162 days.

(21) Appl. No.: **11/776,329**

(22) Filed: **Jul. 11, 2007**

(65) **Prior Publication Data**

US 2008/0032948 A1 Feb. 7, 2008

Related U.S. Application Data

(62) Division of application No. 11/288,807, filed on Nov. 29, 2005, now abandoned, which is a division of application No. 10/297,821, filed as application No. PCT/US01/14860 on Jun. 15, 2001, now Pat. No. 7,053,065.

(60) Provisional application No. 60/215,310, filed on Jun. 30, 2000, provisional application No. 60/235,859, filed on Sep. 27, 2000, provisional application No. 60/284,448, filed on Apr. 18, 2001.

(51) **Int. Cl.**
A61K 31/70 (2006.01)
A61K 31/685 (2006.01)
A61K 31/50 (2006.01)
A61K 31/525 (2006.01)
A61K 31/519 (2006.01)

(52) **U.S. Cl.** **514/52**; 514/77; 514/249; 514/251; 514/265.1

(58) **Field of Classification Search** 514/52, 514/77, 249, 251, 265.1
See application file for complete search history.

(56) **References Cited**

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5,405,839	A	4/1995	Toraya et al.	
5,431,925	A	7/1995	Ohmori et al.	
5,563,126	A	10/1996	Allen et al.	
5,736,402	A	4/1998	Francis et al.	
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WO	WO 95/27723	10/1995	

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(Continued)

Primary Examiner—Kevin Weddington
(74) *Attorney, Agent, or Firm*—Elizabeth A. McGraw

(57) **ABSTRACT**

A method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1001-0001

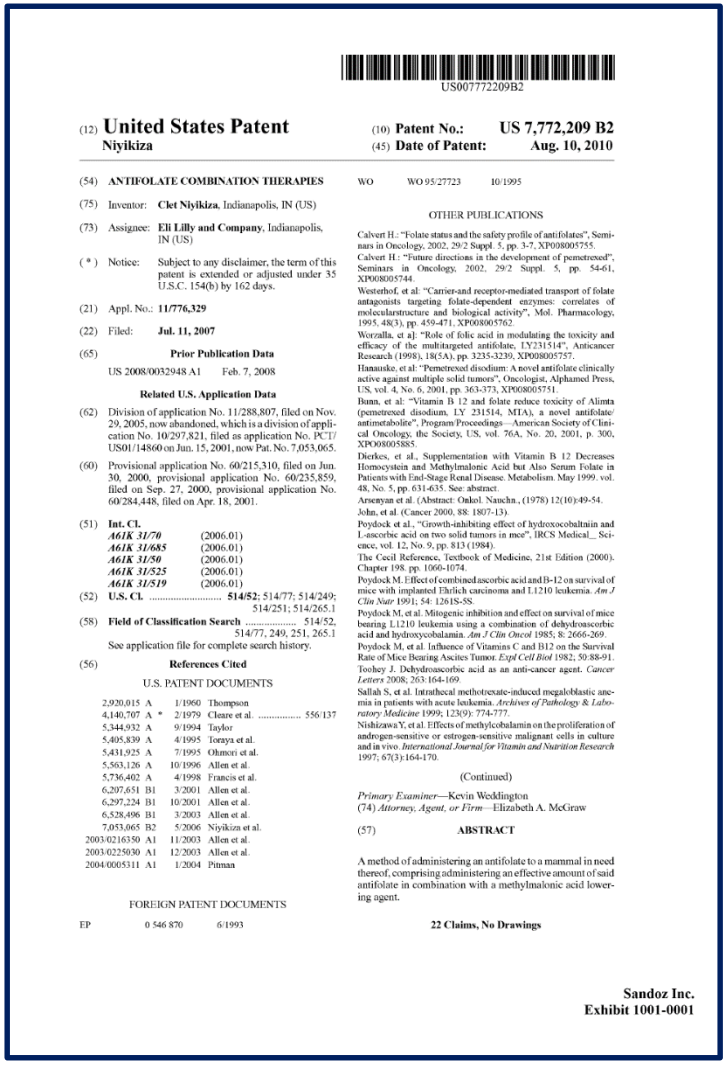
icity of an antifolate in a mammal. The administration of the compounds maybe simultaneous as a single composition or as two separate compositions or can be administered sequentially as separate compositions such that an effective amount of the agent first administered is in the patient's body when the second and/or third agent is administered. The antifolate drug may be administered to the mammal first, followed by treatment with the methylmalonic acid lowering agent. Alter-

In the especially preferred embodiment of this invention, about 0.1 mg to about 30 mg, most preferably about 0.3 mg to about 5 mg, of folic acid is administered orally to a mammal about 1 to 3 weeks post administration of the methylmalonic acid lowering agent and about 1 to about 24 hours prior to the parenteral administration of the amount of an antifolate. However, it will be understood that the amount of the methylmalonic acid lowering agent actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual agent administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect.

Sandoz DX -148

Ex. 1001, '209 patent, col. 4, ll. 9-16, col. 6, ll. 35-47 ; Ex. 1004, Schiff Decl. ¶¶19-20.

Claim Construction of "Patient": Specification



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A method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent.

22 Claims, No Drawings

Sandoz Inc.
Exhibit 1001-0001

Furthermore, the present invention relates to a method of administering an antifolate to a mammal in need thereof, comprising administering an effective amount of said antifolate in combination with a methylmalonic acid lowering agent and a FBP binding agent. A preferred FBP binding agent is folic acid.

Furthermore, the present invention relates to a method of reducing the toxicity associated with the administration of an antifolate to a mammal comprising administering to said mammal an effective amount of said antifolate in combination with a methylmalonic acid lowering agent and a FBP binding agent. A preferred FBP binding agent is folic acid.

Furthermore, the present invention relates to a method of inhibiting tumor growth in mammals comprising administering to said mammals an effective amount of an antifolate in combination with a methylmalonic acid lowering agent and a FBP binding agent. A preferred FBP binding agent is folic acid.

Furthermore, the present invention relates to the use of a methylmalonic acid lowering agent, alone or in combination with a FBP binding agent, in the preparation of a medicament useful in lowering the mammalian toxicity of an antifolate. A preferred FBP binding agent is folic acid.

Furthermore, the present invention relates to the use of a methylmalonic acid lowering agent in the preparation of a medicament useful in lowering the mammalian toxicity associated with an antifolate, and the medicament is administered in combination with an antifolate.

Furthermore, the present invention relates to the use of a methylmalonic acid lowering agent in the preparation of a medicament useful in lowering the mammalian toxicity associated with an antifolate, and the medicament is administered in combination with an antifolate and a FBP binding agent.

Furthermore, the present invention relates to the use of a methylmalonic acid lowering agent in the manufacture of a medicament for use in a method of inhibiting tumor growth in mammals, which method comprises administering said methylmalonic acid lowering agent in combination with an antifolate.

Ex. 1001, '209 patent, col. 3, ll. 1-37; Ex. 1004, Schiff Decl. ¶¶19-20.

Prior Litigation in the S.D. Ind.

Eli Lilly & Co. v. Teva Parenteral Meds., Inc. (Fed. Cir. 2017)

Lilly v. Teva (Fed. Cir.): Folate Combination

Federal Circuit Analysis

To try to overcome this missing link between vitamin B12 deficiency and pemetrexed toxicity, Defendants turn to other prior art references. They argue that, based on those references and perhaps preexisting knowledge, a person of ordinary skill would have known that folate deficiency is correlated with pemetrexed toxicity and that vitamin B12 "directly affect[s] the amount of folate available to healthy cells." Appellants' Opening Br. 45 (citing J.A. 2482, 7894, 7910–11, 8086). As a result, they argue, skilled artisans would have been motivated to use vitamin B12, along with folic acid, to address pemetrexed toxicities. *Id.* Put another way, if we assume that the prior art would have motivated skilled artisans to use folic acid pretreatment to counter pemetrexed toxicity (an issue we do not reach), Defendants submit that those skilled artisans would have also used vitamin B12 as part of the pretreatment because the biochemical pathways for vitamin B12 and folic acid are related. Defendants further submit that other prior art "expressly teaches that folic acid supplementation *improves* the therapeutic index of pemetrexed," so a skilled artisan would not have been concerned about using vitamin B12 supplementation to reduce pemetrexed toxicities. *Id.* at 46.

Slip op. at 26

Lilly v. Teva (Fed. Cir.): B_{12} Combination

Federal Circuit Analysis

But the parties' experts agreed that nothing in the literature as of the critical date described "cancer patients being provided with vitamin B12 supplementation prior to receiving any antifolate," with or without folic acid. J.A. 597–98; *see also* J.A. 1957. Defendants fail to point to evidence that, even if folic acid supplementation were known to improve effects of pemetrexed treatment, a skilled artisan would have thought the same of vitamin B12. Indeed, Eli Lilly offered expert testimony that a skilled artisan would have viewed the use of vitamin B12 with antifolates as "a problem" based on "having to increase the [antifolate] dose to get the same activity" of cancer treatment. J.A. 1453–54.

We are therefore not convinced that the district court committed clear error in concluding that Defendants failed to carry their burden of proving that it would have been obvious to a person of ordinary skill to use vitamin B12 pretreatment to reduce pemetrexed toxicities.

Slip op. at 26-27

Lilly v. Teva (Fed. Cir.): B_{12} Combination (cont'd)

Federal Circuit Analysis (Niyikiza abstracts)

Dr. Niyikiza was an Eli Lilly scientist at the time and is the named inventor on the '209 patent. In 1997, he performed statistical analyses to try to determine which clinical trial patients were likely to develop toxicities from pemetrexed treatment. J.A. 1045, 1071–72. He published the results in the Niyikiza abstracts and reported a correlation between increased pemetrexed toxicities and elevated homocysteine levels. J.A. 7948, 7950–51. Elevated homocysteine levels serve as an indicator of either a folic acid or vitamin B12 deficiency, but they do not indicate which of those two vitamins is specifically lacking. J.A. 622, 719, 7910. Levels of another marker, methylmalonic acid ("MMA"), serve more specifically as an indicator of vitamin B12 deficiency. J.A. 720. But the Niyikiza abstracts reported that "no correlation between toxicity . . . and [MMA levels] was seen." J.A. 7948.

Given the toxicity correlations that Dr. Niyikiza observed with homocysteine levels but not with MMA levels, Eli Lilly's experts testified that the Niyikiza abstracts "present[ed] no evidence for a relationship of vitamin B12 and pemetrexed toxicity" and would not have motivated a skilled artisan to administer vitamin B12 to patients to address pemetrexed toxicity. J.A. 1466–67; *see also* J.A. 1475, 1942. Defendants' expert, Dr. Ratain, confirmed that if a patient exhibits elevated homocysteine but normal MMA levels, a skilled artisan "would conclude that that patient was folate deficient" but "not [vitamin] B12 deficient." J.A. 622–23.

Slip op. at 26-27

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Lilly v. Teva (Fed. Cir.): Dosages

Federal Circuit Analysis

Regarding the dose and schedule of vitamin B12, the district court reiterated that "there are no prior art references where *any amount* of vitamin B_{12} pretreatment had been used with an antifolate in the treatment of cancer." *Eli Lilly II*, 2014 WL 1350129, at *13 (emphasis added). The court also discounted Defendants' citations to literature outside the field of oncology. *Id.* at *13–14.

Defendants argue that, "[o]nce a [skilled artisan] is motivated to use vitamin B12 pretreatment," selecting a dose and schedule for vitamin B12 "would have been routine." Appellants' Opening Br. 47. Setting aside motivation to use vitamin B12 pretreatment in the first instance, Defendants only cite evidence of vitamin B12 doses and schedules that are "routine" in other medical contexts. *See, e.g.*, J.A. 8150, 8169, 756–57. There is no evidence that, considering the context of pemetrexed treatment and associated toxicity problems, a person of ordinary skill would have applied such doses and schedules wholesale.

We therefore also see no clear error in the court's finding that Defendants failed to carry their burden of proving that the prior art disclosed the claimed doses and schedules of vitamin B12 for purposes of pemetrexed pretreatment.

Slip op. at 26-27