Docket No.: 029420.0155-US01
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Alan J. Korman, *et al.*

U.S. Patent No.: 6,984,720

Issued: January 10, 2006

For: HUMAN CTLA-4 ANTIBODIES

TRANSMITTAL LETTER

MS Hatch-Waxman PTE
Commissioner for Patents
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

Dear Sir:

Enclosed are the following items for filing in connection with the above-referenced Patent Application:

1. Fee Transmittal;

2. Request for Extension of Patent Term Under 35 U.S.C. § 156 together with Exhibits 1-8 (original plus two copies);

3. Request for Extension of Patent Term Under 35 U.S.C. § 156 without Exhibits (for fee purposes); and

4. Return receipt postcard.

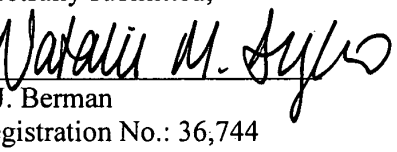
Please charge our Deposit Account No. 50-0740 in the amount of $1,120.00 to cover the required fees. The Director is hereby authorized to charge any deficiency in the fees filed,

asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 50-0740, under Docket No. 029420.0155-US01. A duplicate copy of this paper is attached.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned, and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 50-0740.

Dated: May 16, 2011

Respectfully submitted,

By 

Paul J. Berman
Registration No.: 36,744
Natalie M. Derzko
Registration No.: 48,102
COVINGTON & BURLING LLP
1201 Pennsylvania Avenue, NW
Washington, DC 20004-2401
(202) 662-6000

PTO/SB/17 (10-08)
Approved for use through 09/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person are required to respond to a collection of information unless it displays a valid OMB control number

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2009

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 1,120.00

Complete if Known	
Application Number	Patent No.: 6,984,720
Filing Date	Issued: January 10, 2006
First Named Inventor	Alan J. Korman
Examiner Name	Not Applicable
Art Unit	Not Applicable
Attorney Docket No.	029420.0155-US01

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☒ Deposit Account Deposit Account Number: 50-0740 Deposit Account Name: Covington & Burling LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☐ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fees Paid ($)
Utility	330	165	540	270	220	110	
Design	220	110	100	50	140	70	
Plant	220	110	330	165	170	85	
Reissue	330	165	540	270	650	325	
Provisional	220	110	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	52	26
Each independent claim over 3 (including Reissues)	220	110
Multiple dependent claims	390	195

Total Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 20 or HP =	_____	x _____	= _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 3 or HP =	_____	x _____	= _____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
_____ - 100 =	_____	/50 = _____ (round up to a whole number) x	_____	= _____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): 1457 Extension of term of patent	1,120.00

SUBMITTED BY

Signature	Natalie M. Derzko	Registration No. (Attorney/Agent)	48,102	Telephone	(202) 662-6000
Name (Print/Type)	Natalie M. Derzko			Date	May 16, 2011

Docket No.: 029420.0155-US01
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent of:

Alan J. Korman, et al.

Patent No.: 6,984,720

Issued: January 10, 2006

For: HUMAN CTLA-4 ANTIBODIES

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

REQUEST FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156

Sir:

Pursuant to 35 U.S.C. §156 and 37 C.F.R. §§1.710-1.791, Medarex, Inc., the current address of which is Route 206 and Province Line Road, Princeton, New Jersey 08540 ("Applicant" or "Medarex"), hereby requests an extension of U.S. Patent No. 6,984,720 (the "'720 patent"). As permitted by 37 C.F.R. §1.785(b) and MPEP §2761, Applicant is concurrently filing a request for patent term extension of U.S. Patent No. 7,605,238 based upon the same regulatory review period.

Medarex represents that it is the owner and assignee of the entire interest in and to Letters Patent of the United States No. 6,984,720 (Exhibit 1) granted to Alan J. Korman, Edward L. Halk, Nils Lonberg, Yashwant M. Deo and Tibor P. Keler (the "inventors") on January 10, 2006, for "Human CTLA-4 Antibodies" by virtue of an assignment from the inventors to Medarex, recorded in the United States Patent and Trademark Office ("PTO") on May 18, 2001

at Reel 011817, Frame 0279 (Exhibit 2). The '720 patent matured from U.S. Patent Application No. 09/644,668, filed on August 24, 2000, which claims the benefit of U.S. Provisional Patent Application No. 60/150,452, filed on August 24, 1999, now expired.

The approved product that is relevant to this Request is YERVOY™ (ipilimumab) Injection, for intravenous infusion, referred to herein as "YERVOY" or "Approved Product."

The Marketing Applicant for YERVOY is Bristol-Myers Squibb Company ("BMS'). Medarex is a wholly-owned subsidiary of BMS and is authorized to rely upon the activities of BMS, its predecessors, and affiliates for purposes of this patent term extension application.

The following information is submitted in accordance with 35 U.S.C. §156(d) and the rules for extension of patent term issued by the PTO at 37 C.F.R. Subpart F, §§1.710 to 1.791 and follows the numerical format set forth in 37 C.F.R. §1.740:

(1) A COMPLETE IDENTIFICATION OF THE APPROVED PRODUCT AS BY APPROPRIATE CHEMICAL AND GENERIC NAME, PHYSICAL STRUCTURE OR CHARACTERISTICS:

The approved product is YERVOY, an injection for intravenous infusion of the active ingredient ipilimumab, available in two dosage forms namely, 50 mg/10 ml (5 mg/mL) and 200 mg/40 mL (5 mg/mL). YERVOY has been approved for the treatment of unresectable or metastatic melanoma (approved labeling attached as Exhibit 3). YERVOY is a human cytotoxic T-lymphocyte antigen 4 (CTLA-4)-blocking antibody that is comprised of 1,326 amino acids. The amino acid sequence for YERVOY is as follows.

Antibody Segment	Amino Acid Sequence**
Heavy Chain	
FR1	QVQLVESGGGVVQPGRSLRLSCAASGFTFS
CDR1	SYTMH
FR2	WVRQAPGKGLEWVT
CDR2	FISYDGNNKYYADSVKG
FR3	RFTISRDNSKNTLYLQMNSLRAEDTAIYYCAR
CDR3	TGWLGPFDY
FR4	WGQGTLVTVSS
Heavy Chain Constant Region	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
Light Chain	
FR1	EIVLTQSPGTLSLSPGERATLSC
CDR1	RASQSVGSSYLA
FR2	WYQQKPGQAPRLLIY
CDR2	GAFSRAT
FR3	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
CDR3	QQYGSSPWT
FR4	FGQGTKVEIK
Light Chain Constant Region	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC

** The one-letter amino acid code used in the table follows the nomenclature developed by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochmistry and Molecular Biology (IUB) in the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), "Nomenclature and Symbolism for Amino Acids and Peptides," 1983. See http://www.chem.qmul.ac.uk/iupac/AminoAcid/AA1n2.html#AA1, visited May 13, 2011.

(2) A COMPLETE IDENTIFICATION OF THE FEDERAL STATUTE INCLUDING THE APPLICABLE PROVISION OF LAW UNDER WHICH THE REGULATORY REVIEW OCCURRED:

The Approved Product is a drug product that was approved under section 351 of the Public Health Service Act (PHSA) (42 U.S.C. §262).

(3) AN IDENTIFICATION OF THE DATE ON WHICH THE PRODUCT RECEIVED PERMISSION FOR COMMERCIAL MARKETING OR USE UNDER THE PROVISION OF LAW UNDER WHICH THE APPLICABLE REGULATORY REVIEW PERIOD OCCURRED:

The Approved Product received permission for commercial marketing or use by the United States Food and Drug Administration (FDA) pursuant to section 351(a) of the PHSA (42 U.S.C. § 262(a)) in a letter dated March 25, 2011. A copy of the approval letter is attached as Exhibit 4.

(4) IN THE CASE OF A DRUG PRODUCT, AN IDENTIFICATION OF EACH ACTIVE INGREDIENT IN THE PRODUCT AND AS TO EACH ACTIVE INGREDIENT, A STATEMENT THAT IT HAS NOT BEEN PREVIOUSLY APPROVED FOR COMMERCIAL MARKETING OR USE UNDER THE FEDERAL FOOD, DRUG AND COSMETIC ACT, THE PUBLIC HEALTH SERVICE ACT, OR THE VIRUS-SERUM-TOXIN ACT OR A STATEMENT OF WHEN THE ACTIVE INGREDIENT WAS APPROVED FOR COMMERCIAL MARKETING OR USE (EITHER ALONE OR IN COMBINATION WITH

OTHER ACTIVE INGREDIENTS), THE USE FOR WHICH IT WAS APPROVED, AND THE PROVISION OF LAW UNDER WHICH IT WAS APPROVED: (37 C.F.R. § 1.740(a)(4))

The active ingredient in YERVOY is ipilimumab. Ipilimumab is comprised of 1,326 amino acids and has an amino acid sequence as noted earlier in section (1) of this Request. YERVOY is a human cytotoxic T-lymphocyte antigen 4 (CTLA-4)-blocking antibody that has been approved under section 351(a) of the PHSA for the treatment of unresectable or metastatic melanoma.

Ipilimumab has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the PHSA, or the Virus-Serum-Toxin Act.

(5) A STATEMENT THAT THE APPLICATION IS BEING SUBMITTED WITHIN THE SIXTY DAY PERIOD PERMITTED FOR SUBMISSION PURSUANT TO SECTION 1.720(f) AND AN IDENTIFICATION OF THE DATE OF THE LAST DAY ON WHICH THE APPLICATION COULD BE SUBMITTED:

This Request is timely filed, pursuant to 35 U.S.C. § 156(d)(1), within the permitted sixty-day (60-day) period that began on March 25, 2011, when the product received permission for commercial marketing or use under section 351(a) of the PHSA and that will expire on May 24, 2011. Applicant understands that, pursuant to 37 C.F.R. § 1.720(f), the PTO may deem this period to expire one day earlier, on May 23, 2011.

(6) A COMPLETE IDENTIFICATION OF THE PATENT FOR WHICH AN EXTENSION IS BEING SOUGHT BY THE NAME OF THE INVENTOR, THE PATENT NUMBER, THE DATE OF ISSUE, AND THE DATE OF EXPIRATION:

UNITED STATES PATENT NO.: 6,984,720

INVENTORS: ALAN J. KORMAN
EDWARD L. HALK
NILS LONBERG
YASHWANT M. DEO
TIBOR P. KELER

DATE OF ISSUE: JANUARY 10, 2006

EXPIRATION DATE: AUGUST 2, 2022

The expiration date of U.S. Patent No. 6,984,720 ("the '720 patent") is August 2, 2022, based on the following information. The patent application that issued as the '720 patent, U.S. Patent Application No. 09/644,668 ("the '668 application"), was filed on August 24, 2000, and claims the benefit U.S. Provisional Patent Application No. 60/150,452, filed on August 24, 1999, now expired. Without a patent term adjustment, the expiration of the '720 patent would be August 24, 2020; however, as noted on its face, the '720 patent is entitled to a patent term adjustment pursuant to 35 U.S.C. §154 of 708 days, bringing the expiration of the '720 patent to August 2, 2022.

(7) A COPY OF THE PATENT FOR WHICH AN EXTENSION IS BEING SOUGHT, INCLUDING THE ENTIRE SPECIFICATION (INCLUDING CLAIMS) AND DRAWINGS:

A complete copy of U.S. Patent No. 6,984,720 is attached as Exhibit 1.

(8) A COPY OF ANY DISCLAIMER, CERTIFICATE OF CORRECTION, RECEIPT OF MAINTENANCE FEE PAYMENT, OR RE-EXAMINATION CERTIFICATE ISSUED IN THE PATENT:

No terminal disclaimer or certificate of correction has been filed in U.S. Patent No. 6,984,720 ("the '720 patent"). Moreover, the '720 patent has not been reexamined, and so no re-examination certificate has been issued in U.S. Patent No. 6,984,720.

The first maintenance fee for the '720 patent was paid on July 10, 2009, as shown by the attached Patent Bibliographic Sheet obtained from Public PAIR on May 13, 2011 and the USPTO Maintenance Fee Statement for this patent obtained from Public PAIR on May 13, 2011, both found in Exhibit 5.

Accordingly, there are no unpaid maintenance fees for the '720 patent.

(9) A STATEMENT THAT THE PATENT CLAIMS THE APPROVED PRODUCT, OR A METHOD OF USING OR MANUFACTURING THE APPROVED PRODUCT, AND A SHOWING WHICH LISTS EACH APPLICABLE PATENT CLAIM AND DEMONSTRATES THE MANNER IN WHICH AT LEAST ONE SUCH PATENT CLAIM READS ON THE APPROVED PRODUCT OR A METHOD OF USING OR MANUFACTURING THE APPROVED PRODUCT:

U.S. Patent No. 6,984,720 claims the Approved Product. At least claims 2, 4 and 7, 8 and 10 read on the Approved Product. These claims are set forth below.

> 2. An antibody comprising: (a) a heavy chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:17; and (b) a light chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:7, wherein the antibody is capable of binding human CTLA4.
>
> * * *
>
> 4. An antibody capable of binding human CTLA4, which antibody comprises: (a) a heavy chain variable region having CDR sequences set forth in SEQ ID NOS:27, 32 and 37; and (b) a light chain variable region having CDR sequences set forth in SEQ ID NOS:24, 29 and 35.

* * *

7. An antibody capable of binding human CTLA4, which antibody comprises: (a) a heavy chain variable region of a human V_H 3-30.3 gene; and (b) a light chain variable region of a human V_K A-27 gene.

8. An antibody according to claim 7, wherein the antibody is capable of binding human CTLA4 with a binding affinity of about 10^8 M^{-1} or greater.

* * *

10. An antibody according to claim 7, wherein the antibody inhibits binding of the human CTLA4 to B7-1 or to B7-2.

Pursuant to 37 C.F.R. § 1.740(a)(9), a showing which demonstrates the manner in which one claim reads on the Approved Product is set forth herein below.

CLAIM	The Approved Product
2. An antibody comprising:	Ipilimumab (the active ingredient in YERVOY™) is an antibody.
(a) a heavy chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:17; and	Ipilimumab comprises a heavy chain variable amino acid sequence composed of three complementarity determining regions (CDRs) and four framework regions (FRs) arranged as follows: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4, as set forth immediately under "Heavy Chain" in section (1) of this Request. The amino acid sequence compiled from these regions identified in section (1) of this Request has the amino acid sequence set forth in SEQ ID NO:17 of the '720 Patent.
(b) a light chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:7,	Ipilimumab comprises a light chain variable amino acid sequence composed of three CDRs and four FRs arranged as follows: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4, as set forth immediately under "Light Chain" in section (1) of this Request. The amino acid sequence compiled from these regions

	identified in section (1) of this Request has the amino acid sequence set forth in SEQ ID NO:7 of the '720 patent.
wherein the antibody is capable of binding human CTLA4.	Ipilimumab is capable of binding human CTLA4.

(10) A STATEMENT BEGINNING ON A NEW PAGE OF THE RELEVANT DATES AND INFORMATION PURSUANT TO 35 U.S.C. §156(g) IN ORDER TO ENABLE THE SECRETARY OF HEALTH AND HUMAN SERVICES OR THE SECRETARY OF AGRICULTURE, AS APPROPRIATE, TO DETERMINE THE APPLICABLE REGULATORY REVIEW PERIOD AS FOLLOWS:

(i) FOR A PATENT CLAIMING A HUMAN DRUG, ANTIBIOTIC, OR HUMAN BIOLOGICAL PRODUCT, THE EFFECTIVE DATE OF THE INVESTIGATIONAL NEW DRUG APPLICATION (IND) AND THE IND NUMBER; THE DATE ON WHICH A NEW DRUG APPLICATION (NDA) OR A PRODUCT LICENSE APPLICATION (PLA) WAS INITIALLY SUBMITTED AND THE NDA OR PLA NUMBER; AND THE DATE ON WHICH THE NDA WAS APPROVED OR THE PRODUCTS LICENSE ISSUED:

An original investigational new drug application ("IND") was submitted by Medarex, Inc., now a wholly-owned subsidiary of Bristol-Myers Squibb Co. ("BMS"), on July 12, 2000 and was received by FDA on July 13, 2000. A copy of FDA's acknowledgement letter is provided at Exhibit 6. The FDA assigned BB-IND No. 9186 to this IND, which became effective 30 days after FDA's receipt date, namely, on August 12, 2000.

A biologics license application ("BLA") was submitted by BMS on June 25, 2010 and acknowledged as received on this date in a letter from FDA dated July 8, 2010 (Exhibit 7). The Submission Tracking Number (STN) assigned to this BLA was BL 125377/0. The BLA was approved on March 25, 2011 (Exhibit 4).

(11) A BRIEF DESCRIPTION BEGINNING ON A NEW PAGE OF THE SIGNIFICANT ACTIVITIES UNDERTAKEN BY OWNER, THE MARKETING APPLICANT, DURING THE APPLICABLE REGULATORY REVIEW PERIOD WITH RESPECT TO THE APPROVED PRODUCT AND THE SIGNIFICANT DATES APPLICABLE TO SUCH ACTIVITIES:

In accordance with 37 C.F.R. § 1.740(a)(11), a list of significant activities, undertaken by the Marketing Applicant, its predecessors, and affiliates, in BB-IND No. 9186 and BL 125377/0 during the applicable regulatory review period with respect of the approved product is provided at Exhibit 8.

(12) A STATEMENT BEGINNING ON A NEW PAGE THAT IN THE OPINION OF THE APPLICANT THE PATENT IS ELIGIBLE FOR THE EXTENSION AND A STATEMENT AS TO THE LENGTH OF EXTENSION CLAIMED, INCLUDING HOW THE LENGTH OF EXTENSION WAS DETERMINED:

(a) Statement of the eligibility of the patent for extension under 35 U.S.C. §156(a):

Section 156(a) provides, in relevant part, that the term of a patent which claims a product, a method of using a product, or a method of manufacturing a product shall be extended if (i) the term of the patent has not expired before an application for extension is submitted; (ii) the term of the patent has never been extended under 35 U.S.C. §156(e)(1); (iii) the application for extension is submitted by the owner of record of the patent or its agent in accordance with 35 U.S.C. §156(d); (iv) the product has been subject to a regulatory review period before its commercial marketing or use; and (v) the permission for the commercial marketing or use of the product after such regulatory review period is the first permitted commercial marketing or use of the product using the provision of law under which such regulatory review period occurred.

As described below by corresponding number, each of these elements is satisfied here:

(i) Pursuant to 35 U.S.C. §154, the term of United States Patent No. 6,984,720 is currently set to expire on August 2, 2022. This Request is, therefore, being submitted prior to the expiration of the term of United States Patent No. 6,984,720.

(ii) The term of this patent has never been extended under 35 U.S.C. §156(e)(1).

(iii) This Request is being submitted by Medarex, the owner of record of United States Patent No. 6,984,720. (See Exhibit 2). Medarex is the owner of record by virtue of the duly recorded assignment discussed above. This Request is submitted in accordance with 35 U.S.C. §156(d) in that it is submitted within the sixty-day period beginning on March 25, 2011, the date the product received permission for marketing under section 351 of the Public Health Service Act (PHSA) (42 U.S.C. §262), and ending on May 24, 2011. Moreover, this Request contains the information required under 35 U.S.C. §156(d).

(iv) As evidenced by the March 25, 2011 letter from the FDA to BMS (Exhibit 7), the product was subject to a regulatory review period under section 351 of the Public Health Service Act (PHSA) (42 U.S.C. §262) before its commercial marketing or use.

(v) The permission for the commercial marketing of the YERVOY (ipilimumab) Injection product is the first permitted commercial marketing and use of the product, as defined in 35 U.S.C. §156(f), under section 351 of the Public Health Service Act (PHSA) (42 U.S.C. §262). (See, e.g., Section (4), above.)

(b) Statement as to length of extension claimed.

The term of U.S. Patent No. 6,984,720, now expiring August 2, 2022, should be extended for 966 days, or to March 25, 2025, in accordance with 35 U.S.C. §156.

As set forth in 35 U.S.C. §156(g)(1), the regulatory review period equals the length of time between the effective date of BB-IND No. 9186 of August 12, 2000, and the submission of the BL 125377/0 on June 25, 2010 (i.e., the "testing phase"), a period of 3,604 days, plus the length of time between the submission of the BL 125377/0 on June 25, 2010 to BLA approval on March 25, 2011 (i.e., the "approval phase"), a period of 274 days. These two periods added together equal 3,878 days.

Pursuant to 37 C.F.R. § 1.775(d), the term of the patent as extended is determined by subtracting from the 3,878 day regulatory review period the following:

(i) 1,977 days, which is the number of days in the IND and BLA periods on or before the issuance of U.S. Patent No. 6,984,720 on January 10, 2006; and

(ii) 813 days, which is one-half the number of days remaining in the IND period after the subtraction of 1,977 days above (wherein half days are ignored for purposes of this subtraction, as provided by 37 C.F.R. § 1.775(d)(1)(iii)).

From the foregoing calculation, an extension of 1,087 days results, i.e., the remaining period under 35 U.S.C. §156(g)(1)(B)(i) (813 days) plus the remaining period under 35 U.S.C. §156(g)(1)(B)(ii) (274 days). This length of an extension would provide a new expiration date for U.S. Patent No. 6,984,720 of July 24, 2025. However, this extension period is subject to two further potential limitations under 35 U.S.C. §156. One of these potential limitations does further limit the term of the patent and the other does not.

First, under 35 U.S.C. §156(g)(6)(A), a maximum extension of five years is permitted (i.e., 1826 days in this case). Since the current expiry date of U.S. Patent No. 6,984,720 is August 2, 2022, no patent term extension could extend the term of the patent beyond August 2, 2027. Consequently, this provision does not operate to limit the possible extension available to U.S. Patent 6,984,720.

Second, under 35 U.S.C. §156(c)(3), the calculated extension period cannot lead to a patent term that would result in a patent term exceeding 14 years after the date of approval, that is, a patent term expiring after March 25, 2025. In this case, 35 U.S.C. §156(c)(3) does operate to limit the possible extension available to U.S. Patent 6,984,720. The period from August 2, 2022 to March 25, 2025 amounts to 966 days.

Accordingly, United States Patent No. 6,984,720 is eligible for a patent term extension of 966 days.

(13) A STATEMENT THAT APPLICANT ACKNOWLEDGES A DUTY TO DISCLOSE TO THE COMMISSIONER OF PATENTS AND TRADEMARKS AND THE SECRETARY OF HEALTH AND HUMAN SERVICES ANY INFORMATION WHICH IS MATERIAL TO THE DETERMINATION OF ENTITLEMENT TO THE EXTENSION SOUGHT (SEE 37 C.F.R. §1.765).

Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

In accordance with the duty of disclosure described in 37 C.F.R. § 1.765 and acknowledged under 37 C.F.R. § 1.740(13), Applicant wishes to inform the Office that two patent term extension applications have been filed concurrently with respect to the regulatory review period for YERVOY™ (ipilimumab) Injection. Such patent term extension applications are with respect to U.S. Patent No. 6,984,720 (i.e., the present application) and U.S. Patent No. 7,605,238. It is requested that the Office examine these extension applications concurrently so that a meaningful election can be made upon the receipt of a Notice of Final Determination and Requirement of Election as to which patent to ultimately extend in accordance with 37 C.F.R. § 1.785.

(14) THE PRESCRIBED FEE FOR RECEIVING AND ACTING UPON THE APPLICATION FOR EXTENSION (SEE 37 C.F.R. §1.20(j)):

Please charge our Deposit Account No. 50-0740 in the amount of $1,120.00 to cover the fee for a request for extension of patent term. The Director is hereby authorized to charge our Deposit Account No. 50-0740, under Docket No. 029420.00155, for any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm), to prevent this application from being inadvertently abandoned. A duplicate of this Request (without Exhibits 1-8) is attached.

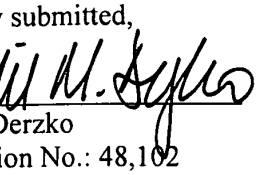
(15) THE NAME, ADDRESS, AND TELEPHONE NUMBER OF THE PERSON TO WHOM INQUIRIES AND CORRESPONDENCE RELATING TO THE APPLICATION FOR PATENT TERM EXTENSION ARE TO BE DIRECTED:

Natalie M. Derzko
COVINGTON & BURLING LLP
1201 Pennsylvania Avenue, N.W.
Washington, DC 20004-2401
Telephone No.: (202) 662-6000
Facsimile No.: (202) 662-6291

Pursuant to 37 C.F.R. §1.740(b), this Request for Extension of Patent Term Under 35 U.S.C. §156, including Exhibits 1-8, is accompanied by two additional copies, for a total submission of three copies.

Dated: May 16, 2011

Respectfully submitted,

By 

Natalie M. Derzko
Registration No.: 48,102
Paul J. Berman
Registration No.: 36,744
COVINGTON & BURLING LLP
1201 Pennsylvania Avenue, N.W.
Washington, DC 20004-2401
(202) 662-6000
Attorneys for Applicant

Exhibit A

US006984720B1

(12) **United States Patent**
Korman et al.

(10) **Patent No.: US 6,984,720 B1**
(45) **Date of Patent: Jan. 10, 2006**

(54) **HUMAN CTLA-4 ANTIBODIES**

(75) Inventors: **Alan J. Korman**, Piedmont, CA (US); **Edward L. Halk**, Sunnyvale, CA (US); **Nils Lonberg**, Woodside, CA (US); **Yashwant M. Deo**, Annandale, NJ (US); **Tibor P. Keler**, Ottsville, PA (US)

(73) Assignee: **Medarex, Inc.**, Annandale, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 708 days.

(21) Appl. No.: **09/644,668**

(22) Filed: **Aug. 24, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/150,452, filed on Aug. 24, 1999.

(51) **Int. Cl.**

C12P 21/08	(2006.01)
C07K 16/00	(2006.01)
C07K 16/28	(2006.01)

(52) **U.S. Cl.** **530/388.22**; 530/387.1; 530/387.9; 530/388.1

(58) **Field of Classification Search** 530/387.1, 530/387.9, 388.1, 388.22, 388.15, 388.2, 530/388.7, 388.73, 388.75, 387.3
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,399,216	A	8/1983	Axel et al.
4,681,581	A	7/1987	Coates
4,683,195	A	7/1987	Mullis et al.
4,683,202	A	7/1987	Mullis
4,735,210	A	4/1988	Goldenberg
4,740,461	A	4/1988	Kaufman
4,816,397	A	3/1989	Boss et al.
4,921,040	A	5/1990	Ueruenduel et al.
4,959,455	A	9/1990	Clark et al.
5,101,827	A	4/1992	Goldenberg
5,151,510	A	9/1992	Stec et al.
5,194,594	A	3/1993	Khawli et al.
5,434,131	A	7/1995	Linsley et al.
5,530,101	A	6/1996	Queen et al.
5,545,806	A	8/1996	Lonberg et al.
5,545,807	A	8/1996	Surani et al.
5,569,825	A	10/1996	Lonberg et al.
5,585,089	A	12/1996	Queen et al.
5,591,669	A	1/1997	Krimpenfort et al.
5,612,205	A	3/1997	Kay et al.
5,625,126	A	4/1997	Lonberg et al.
RE35,500	E	5/1997	Rhodes
5,633,425	A	5/1997	Lonberg et al.
5,643,763	A	7/1997	Dunn et al.
5,648,471	A	7/1997	Buttram et al.
5,661,016	A	8/1997	Lonberg et al.
5,693,761	A	12/1997	Queen et al.
5,693,792	A	12/1997	Torii et al.
5,697,902	A	12/1997	Goldenberg
5,703,057	A	12/1997	Johnston et al.
5,714,350	A	2/1998	Co et al.
5,721,367	A	2/1998	Kay et al.
5,733,743	A	3/1998	Johnson et al.
5,741,957	A	4/1998	Deboer et al.
5,750,172	A	5/1998	Meade et al.
5,756,687	A	5/1998	Denman et al.
5,770,197	A	6/1998	Linsley et al.
5,770,429	A	6/1998	Lonberg et al.
5,773,253	A	6/1998	Linsley et al.
5,777,085	A	7/1998	Co et al.
5,789,215	A	8/1998	Berns et al.
5,789,650	A	8/1998	Lonberg et al.
5,811,097	A	9/1998	Allison et al.
5,814,318	A	9/1998	Lonberg et al.
5,827,690	A	10/1998	Meade et al.
5,844,095	A	12/1998	Linsley et al.
5,855,887	A	1/1999	Allison et al.
5,874,299	A	2/1999	Lonberg et al.
5,877,397	A	3/1999	Lonberg et al.
5,885,796	A	3/1999	Linsley et al.
5,916,771	A	6/1999	Hori et al.
5,939,598	A	8/1999	Kucherlapati et al.
5,968,510	A	10/1999	Linsley et al.
5,977,318	A	11/1999	Linsley et al.
6,051,227	A	4/2000	Allison et al.
6,075,181	A	6/2000	Kucherlapati et al.
6,114,598	A	9/2000	Kucherlapati et al.
6,150,584	A	11/2000	Kucherlapati et al.
6,162,963	A	12/2000	Kucherlapati et al.
6,207,156	B1	3/2001	Kuchroo et al.
6,255,458	B1	7/2001	Lonberg et al.
6,682,736	B1 *	1/2004	Hanson et al. 424/144.1
2002/0086014	A1	7/2002	Korman et al.

FOREIGN PATENT DOCUMENTS

CA	2205680	B1	11/1998
EP	0 256 055	B1	8/1991
EP	0 323 997	B1	4/1993
EP	0 338 841	B1	3/1995
EP	0 216 846	B2	4/1995
EP	0 463 151	B1	6/1996
EP	0 546 073	B1	9/1997
WO	WO 90/04036	*	4/1990
WO	WO91/10741		7/1991

(Continued)

OTHER PUBLICATIONS

L. Green et al.: "Regulation of B Cell Development by Variable Gene Complexity in Mice Reconstituted with Human Immunoglobulin Yeast Artificial Chromosome," J. Exp. Med., vol. 188, No. 3, Aug. 3, 1998 483–495.

(Continued)

Primary Examiner—Phillip Gambel
Assistant Examiner—Ilia Ouspenski
(74) *Attorney, Agent, or Firm*—Darby & Darby

(57) **ABSTRACT**

The present invention provides human sequence antibodies against CTLA-4 and methods of treating human diseases, infections and other conditions using these antibodies.

14 Claims, 20 Drawing Sheets

FOREIGN PATENT DOCUMENTS

WO	WO92/03918		3/1992
WO	WO92/22645		12/1992
WO	WO 92/22647	A1	12/1992
WO	WO 92/22670	A1	12/1992
WO	WO 93/00431	A1	1/1993
WO	WO93/12227		6/1993
WO	WO94/02602		2/1994
WO	WO94/25585		11/1994
WO	WO 94/29444	A1	12/1994
WO	WO 95/01994	A1	1/1995
WO	WO 95/03408	A1	2/1995
WO	WO 95/24217	A1	9/1995
WO	WO95/33770		12/1995
WO	WO 96/14436	A1	5/1996
WO	WO 96/22380	A2	7/1996
WO	WO96/33735		10/1996
WO	WO96/34096		10/1996
WO	WO97/07671		3/1997
WO	WO 97/13852	A1	4/1997
WO	WO 97/20574	A1	6/1997
WO	WO 97/38137	A1	10/1997
WO	WO98/24884		6/1998
WO	WO98/24893	A3	6/1998
WO	WO98/24893	A2	6/1998
WO	WO98/37757		9/1998
WO	WO98/42752		10/1998
WO	WO 98/46996	A2	10/1998
WO	WO 98/50433	A2	11/1998
WO	WO 00/00569	A1	1/2000
WO	WO00/10383		3/2000
WO	WO 00/32231	A1	6/2000
WO	WO00/37504		6/2000
WO	WO 01/14424	A2	3/2001

OTHER PUBLICATIONS

L. Green et al.: et al.: "Antigen-specific human monoconal antibodies from mice engineered with human Ig heavy and light chain YACs," Nature Genetics, vol. 7, May 1994.

T. Choi et al.: "Transgenic mice containing a human heavy chain immunoglobulin gene fragment cloned in a yeast artificial chromosome," Nature Genetics, vol. 4, Jun. 1993.

N. Tuaillon et al.: "Human immunoglobulin heavy-chain minilocus recombination in transgenic mice: Gene-segment use in μ and γ transcripts," Proc. Natl. Acad. Sci. USA., vol. 99, pp. 3720-3724, Apr. 1993, Immunology.

N. Tuaillon et al.: "Analysis of Direct and Inverted DJ_h Rearrangements in a Human Ig Heavy Chain Transgenic Minilocus," The Journal of Immunology, 1995, 154: 6453-6456.

L. Taylor et al.: "Human immunoglobulin transgenes undergo rearrangment, somatic mutation and class switching in mice that lack endogenous IgM," International Immunology, vol. 6, No. 4, pp. 579-591, 1994.

N. Lonberg et al.: "Antigen-specific human antibodies from mice comprising four distinct genetic modifications," Nature, vol. 368, Apr. 28, 1994.

M. Mendez et al.: "Functional transplant ofmegabase human immunoglobullin loci recapitulates human antibody response in mice," Nature Genetics, vol. 15, Feb. 1997.

L. Taylor et al.: "A transgenic mouse that expresses a diversity of human sequence heavy and light chain immunoglobulins," Nucleic Acids Research, 1992, vol. 20, No. 23, pp. 6287-6295.

J. Chen et al.: "Immunoglobulin gene rearrangement in B cell deficient mice generated by targeted deletion of the Jh locus," International Immunology, vol. 5, No. 6, pp. 647-656, 1993.

Blair et al. "Cutting edge: CTLA-4 ligation delivers a unique signal to resting human CD4 T cells that inhibits interleukin-2 secretion but allows Bcl-X_L induction," The J. of Immunology 22:1767 (1998).

Bluestone (1997) J. Immunol. 158:1989-1993.

Brunet et al., Immunol. Rev. 103-21-36 (1988).

Brunet et al., Nature 328:267-270(1987).

Chambers (1997) Curr. Opin. Immunol. 9:396-404.

Chambers et al., Immunity. 7:885-895(1997).

Clark (1986) Human Immunol. 16:100-113.

Damle et al., Proc. Natl. Acad. Sci. 78:5096-6001 (1981).

Dariavach et al., Eur. J. Immunol. 18:1901-1905 (1988).

Elsas et al. "Combination Immunotherapy of B16 melanoma using anti-cytoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation," J. Exp. Med. 190:355-366 (1999).

Fishwild et al. "High-avidity human IgGk monoclonal antibodies from a novel strain of minilocus mice," Nature Biotechnology 14:845-851 (1996).

Freedman (1987) J. Immunol. 138:3260-3267.

Freeman (1989) J. Immunol. 143:2714-2722.

Gribben et al. "CTLA4 mediates antigen-specific apoptosis of human T cells," Proc. Natl. Acad. Sci. USA 92:811-815 (1995).

Hurwitz et al. "CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma," Proc. Natl. Acad, Sci. USA 95:10067-10071 (1998).

Hurwitz et al. "Immunotherapy of primary prostrate cancer in a transgenic model using a combination of CTLA-4 blockade and tumor cell vacine," Cancer Research 60:2444-2448 (2000).

Kearney (1995) J. Immunol. 155:1032-1036.

Kohno et al., Cell Immunol. 131-1-10 (1990).

Krummel et al. "CD28 and CTLA-4 have opposing effects on the responses of T cells to stimulation," (1995) J. Exp. Med. 182:459-465).

Krummel et al., "CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells," J. Exp. Med 183:2533-2540 (1996).

Krummel et al. "Superantigen responses and co-stimulation: CD28 and CTLA-4 have opposing effects on T cell expansion in vitro and in vivo," (1996) Int'l Immunol. 8:519-523.

Kwon et al. "Manipulation of T cell costimulatory and inhibitory signals for immunotherapy of prostrate cancer," proc. Natl. Acad, Sci. USA 94:8099-8103 (1997).

Lafage-Pochitaloff et al., Immunogenetics 31:198-201 (1990).

Leach et al. "Enhancement of antitumor immunity by CTLA-4 blockade," Science 271:1734-1736 (1996).

Lesslauer et al., Eur. J. Immunol. 16:1289-1296 (1986).

Lindsten et al., Science 244:339-343 (1989).

Linsley et al. "Coexpression and functional cooperation of CTLA-4 and CD28 on activated T lymphocytes," J Exp. Med. 176:1595-1604 (1992).

Linsley et al. "CTLA-4 is a second receptor for the B cell activation antigen B7," J. Exp. Med. 174:561-569 (1991).

Linsley et al., J. Exp. Med. 173:721–730 (1991).
Linsley et al., Proc. Natl. Acad. Sci. USA 87:5031–5035 (1990).
Lonberg et al. "Human antibodies from transgenic mice," International Review of Immunology 13:65–93 (1995).
Luhder (1998) J Exp. Med. 187:427–432.
Matsui (1999) J. Immunol. 162:4328–4335.
McCoy et al. "Protective immunity to nematode infection is induced by CTLA–4 blockade," J. Exp. Med 186:183–187 (1997).
Neuberger "Generating high–avidity human Mabs in mice," Nature Biotechnology 14:826 (1996).
Thompson (1997) Immunity 7:445–450.
Thompson et al., Proc. Natl. Acad. Sci 86:1333–1337 (1989).
Walunas et al. "CTLA–4 ligation blocks CD28–dependant T cell activation," J. Exp. Med 183:2541–2550 (1996).
Walunas, et al., "CTLA–4 can function as a negative regulator of T Cell activation," Immunity. 1:405–413 (1994).
Wu et al., J Exp. Med. 185:1327–1335 (1997).
Yang et al. "Eradication of established tumors by a fully human monoclonal antibody to the epidermal growth factor receptor without concomitant chemotherapy," Cancer Research 59:1236–1243 (1999).
Yokochi (1981) J. Immunol. 128:823.

* cited by examiner

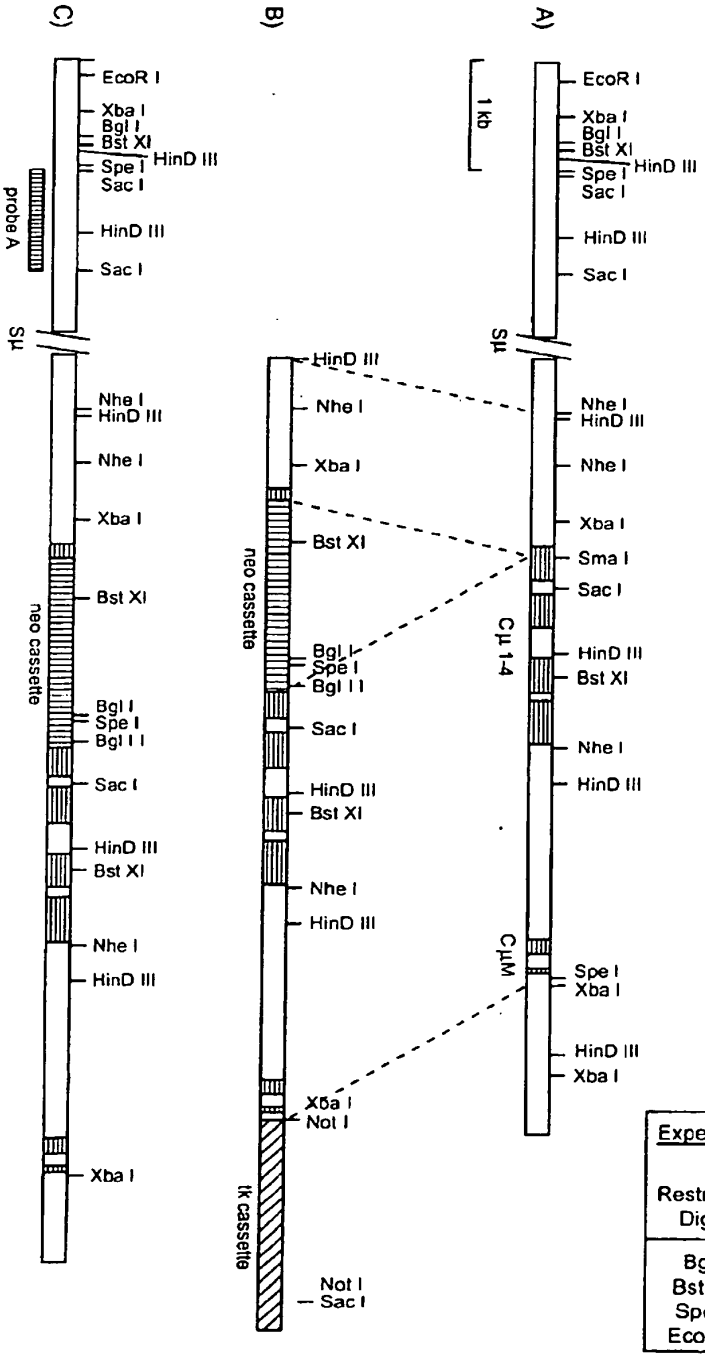


FIG. 1

Expected Fragment Sizes (kb) using Probe A		
Restriction Digest	Fragment Length wild type	mutant
BglI	15.7	7.7
Bst XI	7.3	6.6
Spe I	9.9	7.6
Eco RI	12.5	14.3

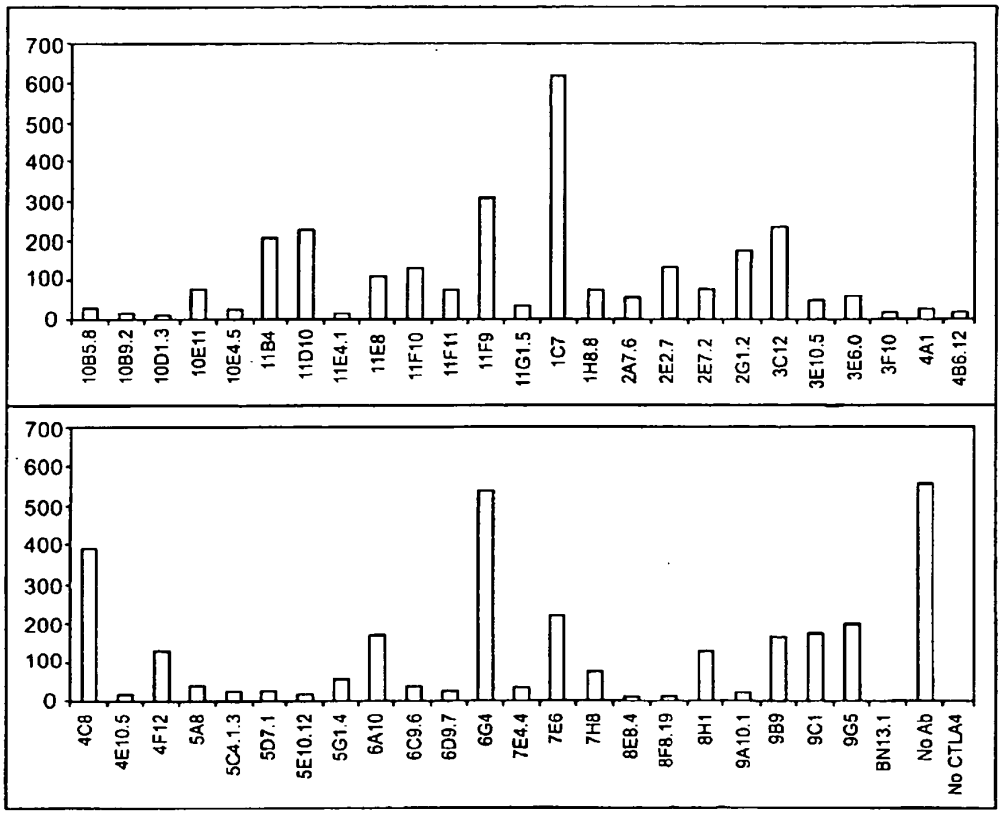

700
600
500
400
300
200
100
0
10B5.8
10B9.2
10D1.3
10E11
10E4.5
11B4
11D10
11E4.1
11E8
11F10
11F11
11F9
11G1.5
1C7
1H8.8
2A7.6
2E2.7
2E7.2
2G1.2
3C12
3E10.5
3E6.0
3F10
4A1
4B6.12
700
600
500
400
300
200
100
0
4C8
4E10.5
4F12
5A8
5C4.1.3
5D7.1
5E10.12
5G1.4
6A10
6C9.6
6D9.7
6G4
7E4.4
7E6
7H8
8E8.4
8F8.19
8H1
9A10.1
9B9
9C1
9G5
BN13.1
No Ab
No CTLA4

FIG. 2

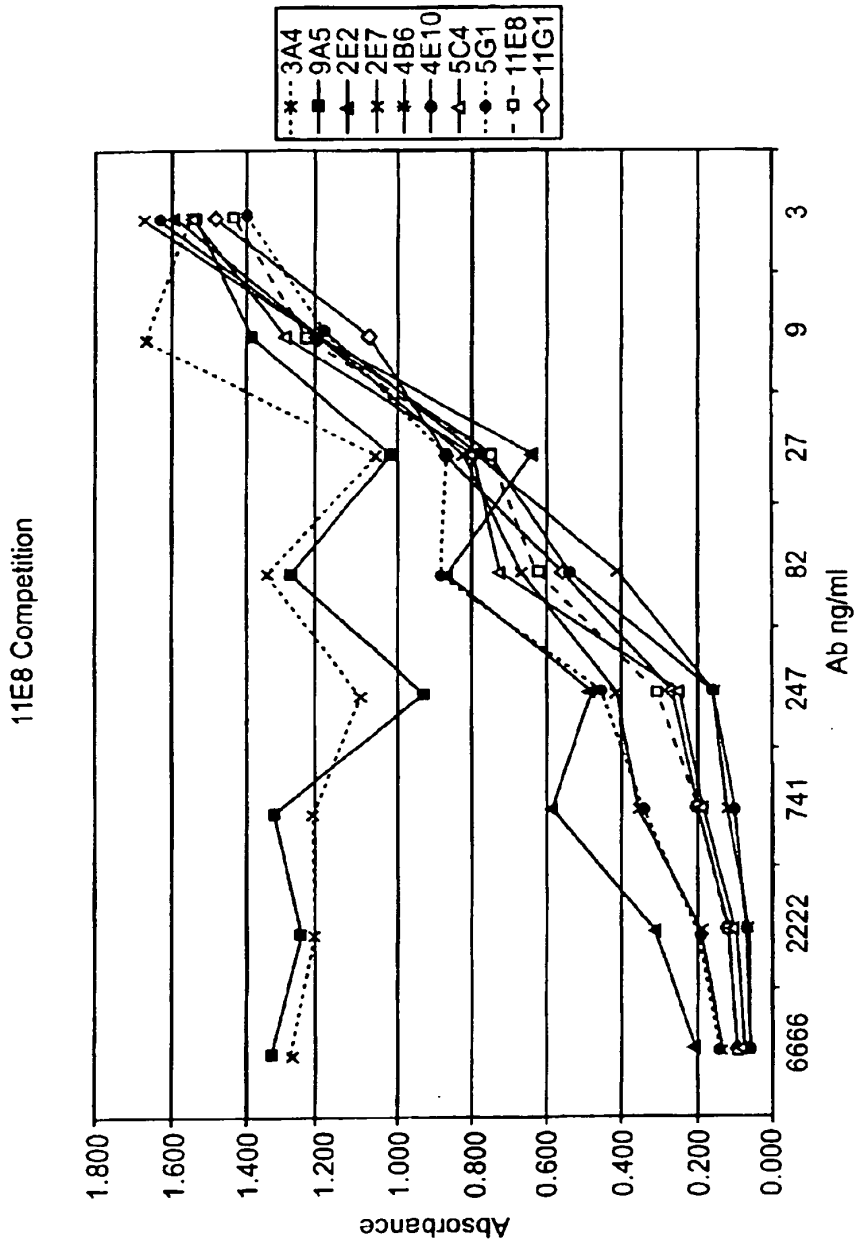

3A4
9A5
2E2
2E7
4B6
4E10
5C4
5G1
11E8
11G1
11E8 Competition
Absorbance
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Ab ng/ml
3
9
27
82
247
741
2222
6666

FIG. 3

10D1.3 VH (SEQ ID NO:2)

```
TGGGGGAGGC GTGGTCCAGC CTGGGAGGTC CCTGAGACTC TCCTGTGCAG  50
CCTCTGGATT CACCTTCAGT AGCTATACTA TGCACTGGGT CCGCCAGGCT 100
CCAGGCAAGG GGCTGGAGTG GGTGACATTT ATATCATATG ATGGAAACAA 150
TAAATACTAC GCAGACTCCG TGAAGGGCCG ATTCACCATC TCCAGAGACA 200
ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGAG AGCTGAGGAC 250
ACGGCTATAT ATTACTGTGC GAGGACCGGC TGGCTGGGGC CCTTTGACTA 300
CTGGGGCCAG GGAACCCTGG TCACCGTCTC CTCAGCCTCC ACCAAGGGC  349
```

10D1.3 VK (SEQ ID NO:3)

```
CTCCAGGCAC CCTGTCTTTG TCTCCAGGGG AAAGAGCCAC CCTCTCCTGC  50
AGGGCCAGTC AGAGTGTTGG CAGCAGCTAC TTAGCCTGGT ACCAGCAGAA 100
ACCTGGCCAG GCTCCCAGGC TCCTCATCTA TGGTGCATTC AGCAGGGCCA 150
CTGGCATCCC AGACAGGTTC AGTGGCAGTG GGTCTGGGAC AGACTTCACT 200
CTCACCATCA GCAGACTGGA GCCTGAAGAT TTTGCAGTGT ATTACTGTCA 250
GCAGTATGGT AGCTCACCGT GGACGTTCGG CCAAGGGACC AAGGTGGAAA 300
TCAAACGAAC TGTGGCTGCA C                                 321
```

FIG. 4

SEQ ID NOs:4,6&8 (respectively)

```
VK A-27
Germline:  GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC
10D1:      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
4B6:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

           ____________________CDR1_______________________
VK A-27:   AGG GCC AGT CAG AGT GTT AGC AGC AGC TAC TTA GCC TGG TAC CAG CAG AAA CCT GGC CAG GCT CCC AGG
10D1:      --- --- --- --- --- --- G-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
4B6:       --- --- --- --- --- --- --- --- --- -T- --- --- --- --- --- --- --- --- --- --- --- --- ---

                               ___________CDR2____________
VK A-27:   CTC CTC ATC TAT GGT GCA TCC AGC AGG GCC ACT GGC ATC CCA GAC AGG TTC AGT GGC AGT GGG TCT GGG
10D1:      --- --- --- --- --- --- -T- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
4B6:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

VK A-27:   ACA GAC TTC ACT CTC ACC ATC AGC AGA CTG GAG CCT GAA GAT TTT GCA GTG TAT TAC TGT
10D1:      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
4B6:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

           _______________CDR3_______________
                                      Jκ1
VK A-27:   CAG CAG TAT GGT AGC TCA CC
10D1:      --- --- --- --- --- --- --G TGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA C/
4B6:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
```

SEQ ID NOs:10&12 (respectively)

```
VK L-15
Germline:  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT
1E2:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

           __________________CDR1____________________
VK L-15:   CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC
1E2:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                       _________CDR2______________
VK L-15:   CTG ATC TAT GCT GCA TCC AGT TTG CAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA
1E2:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

FIG. 5
1 of 2

```
                                                                                                      CDR3-
VK L-15:  GAT TTC ACT CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG TAT AAT
1E2:      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                     Jκ1
VK L-15:  AGT TAC CCT CC
1E2:      --- --- --- --G ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA C/
```

FIG. 5
2 of 2

SEQ ID NOs:14,16&18 (respectively)

```
VH 3-30.3
Germline:  CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG AGA CTC TCC TGT GCA GCC
10D1:      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
4B6:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                        CDR1
VH 3-30.3: TCT GGA TTC ACC TTC AGT AGC TAT GCT ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
10D1:      --- --- --- --- --- --- --- --- A-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
4B6:       --- --- --- --- --- --- --- --- A-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                                    CDR2
VH 3-30.3: GCA GTT ATA TCA TAT GAT GGA AGC AAT AAA TAC TAC GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA
10D1:      A-- T-- --- --- --- --- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
4B6:       A-- T-- --- --- --- --- --- --- --- --- C-- --- --- --- --- --- --- --- --- --- --- G-- --- ---

VH 3-30.3: GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT
10D1:      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- A-A --- ---
4B6:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- A-A --- ---

                                CDR3
                                D7-27    J_H4b
VH 3-30.3: GCG AGA
10D1:      --- --G ACC GGC TGG CTG GGG CCC TTT GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA G/
4B6:       --- --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -/
```

SEQ ID NOs:20&22 (respectively)

```
VH 3-33
Germline:  CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG AGA CTC TCC TGT GCA GCG
1E2:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                        CDR1
VH 3-33:   TCT GGA TTC ACC TTC AGT AGC TAT GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
1E2:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                                    CDR2
VH 3-33:   GCA GTT ATA TGG TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA
1E2:       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

FIG. 6
1 of 2

```
VH 3-33:  GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACG GCT GTG TAT TAC TGT
1E2:      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -T- --- ---

                    _____________CDR3______________
                                  JH3b
VH 3-33:  GCG AGA GA
1E2:      --- --- -CT CCC AAT TAT ATT GGT GCT TTT GAT GTC TGG GGC CAA GGG ACA ATG GTC ACC GTC TCT TCA G/
```

FIG. 6
2 of 2

SEQ ID NOs:5,7&9 (respectively)

		CDR1		CDR2
VK A-27 Germline:	EIVLTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT
10D1:	----------	------G-----	----------	--F----
4B6:	----------	---------F--	----------	-------

		CDR3		
VK A-27:	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSS		
10D1:	----------	------PWT	FGQGTKVEIK	
4B6:	----------	---------	----------	

SEQ ID NOs:11&13 (respectively)

		CDR1		CDR2
VK L-15 Germline:	DIQMTQSPSSLSASVGDRVTITC	RASQGISSWLA	WYQQKPEKAPKSLIY	AASSLQS
1E2:	----------	----------	----------	-------

		CDR3		
VK L-15:	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQYNSY		
1E2:	----------	------PPT	FGQGTKVEIK	

FIG. 7

SEQ ID NOs:15,17&19 (respectively)

		CDR1		CDR2
VH 3-30.3 Germline:	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYAMH	WVRQAPGKGLEWVA	VISYDGSNKYYADSVKG
10D1:	----------	--T--	----------T	F-----N----------
4B6:	----------	--T--	----------T	F--------H-------

		CDR3		
VH 3-30.3:	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR			
10D1:	----------I-----	TGWLGPFDY	WGQGTLVTVSS	
4B6:	---V----------I-----	---------	----------	

SEQ ID NOs:21&23 (respectively)

		CDR1		CDR2
VH 3-33 Germline:	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH	WVRQAPGKGLEWVA	VIWYDGSNKYYADSVKG
1E2:	----------	-----	----------	----------

		CDR3		
VH 3-33:	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR			
1E2:	----------F----	APNYIGAFDV	WGQGTMVTVSS	

FIG. 8

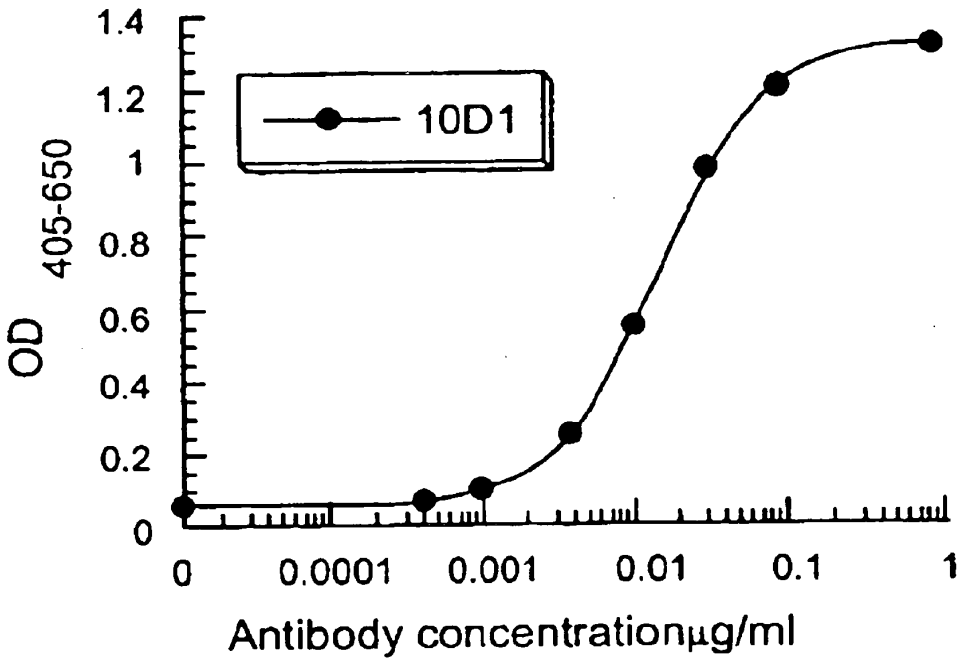

1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 405-650
10D1
0
0.0001
0.001
0.01
0.1
1
Antibody concentrationμg/ml

FIG. 9

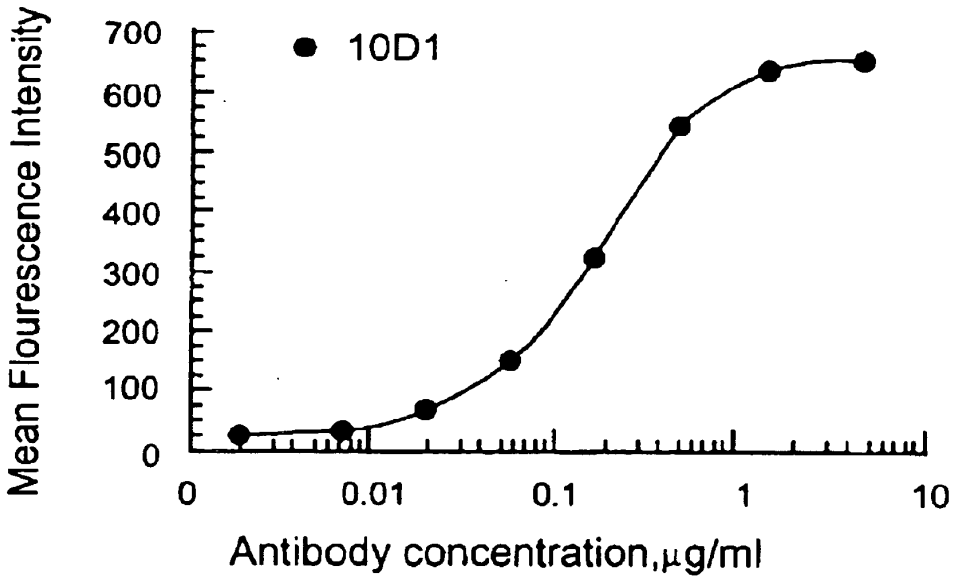

Mean Flourescence Intensity
700
600
500
400
300
200
100
0
10D1
0
0.01
0.1
1
10
Antibody concentration,μg/ml

FIG. 10

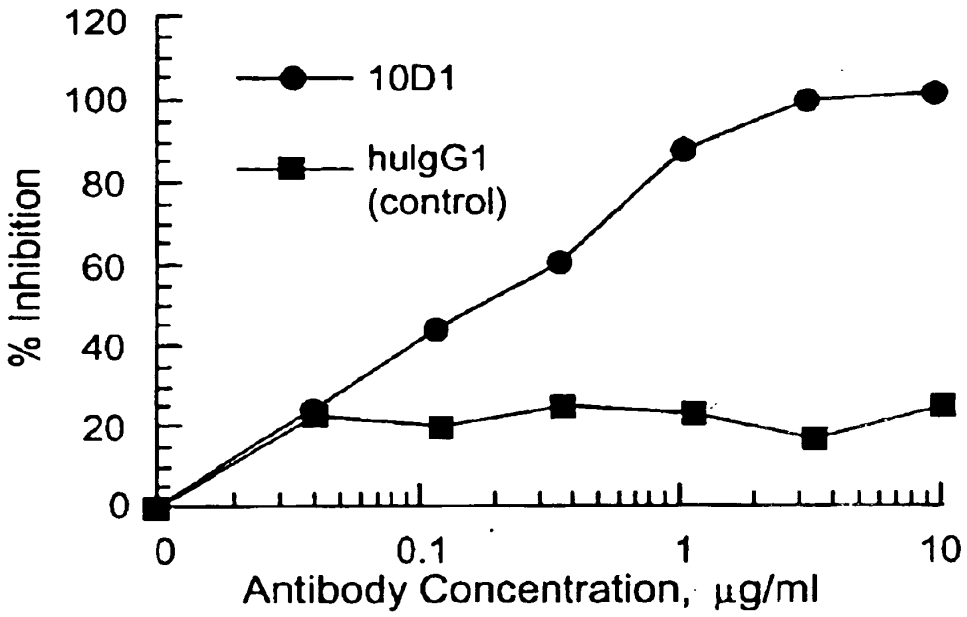

120
100
80
60
40
20
0
% Inhibition
10D1
hulgG1
(control)
0
0.1
1
10
Antibody Concentration, µg/ml

FIG. 11

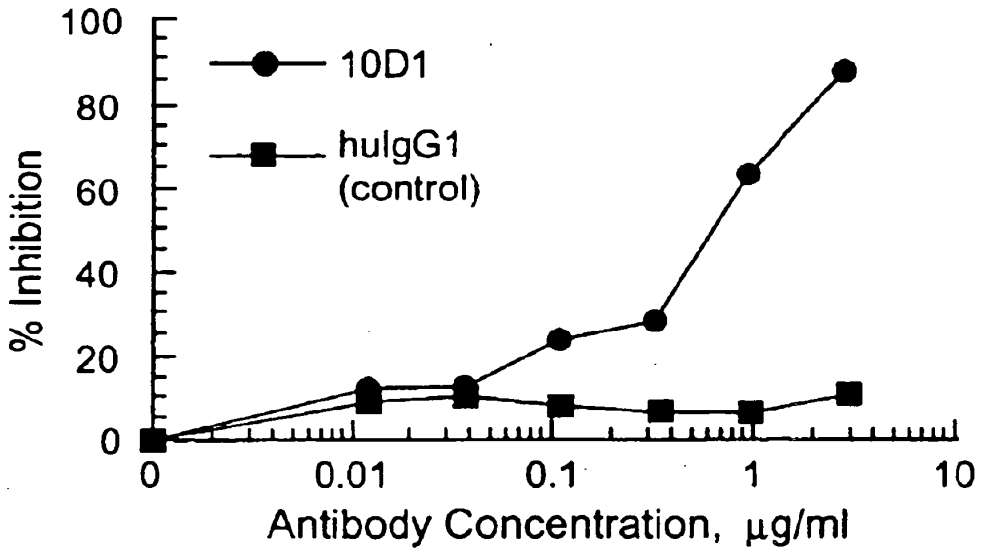

100
80
60
40
20
0
% Inhibition
10D1
huIgG1
(control)
0
0.01
0.1
1
10
Antibody Concentration, µg/ml

FIG. 12

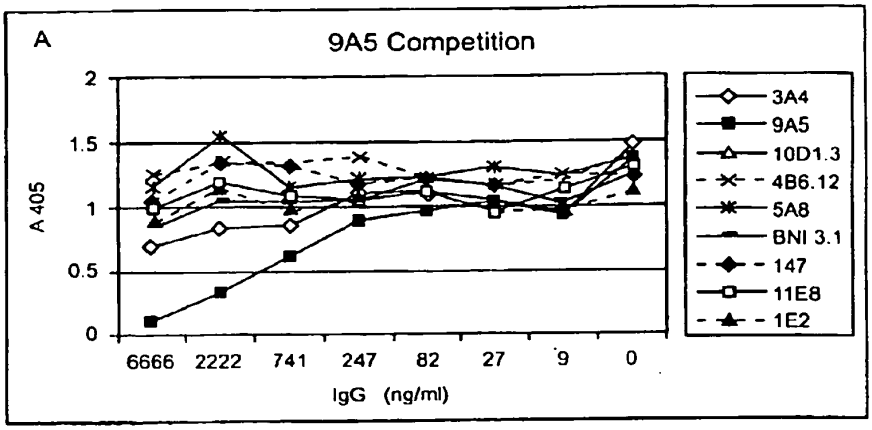


FIG. 13A

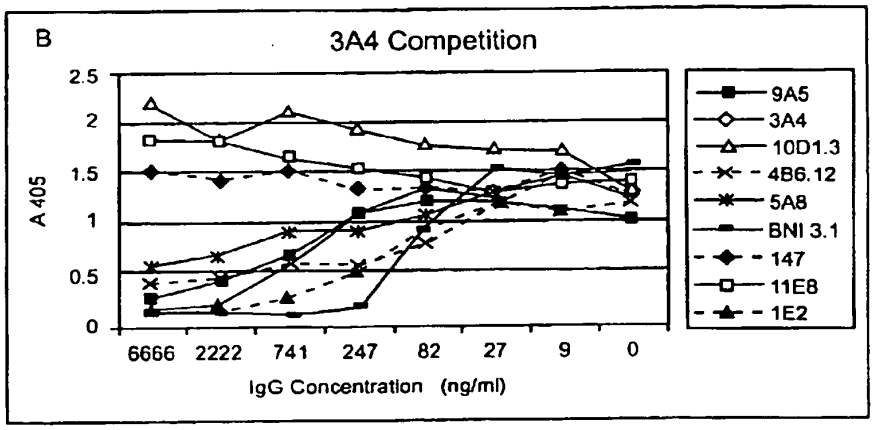


FIG. 13B

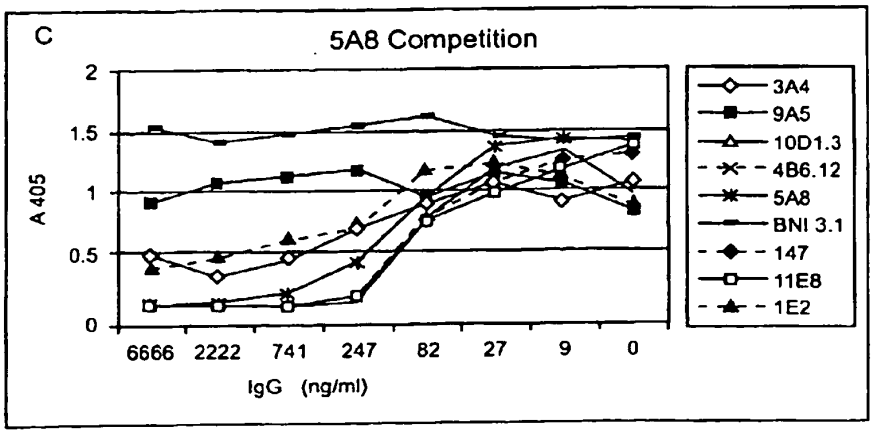


FIG. 13C

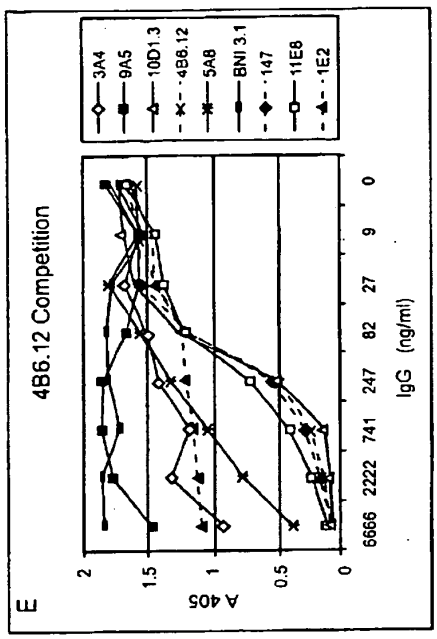


FIG. 13E

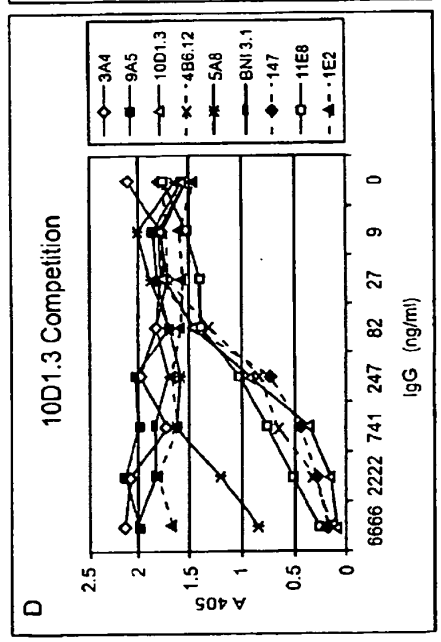


FIG. 13D

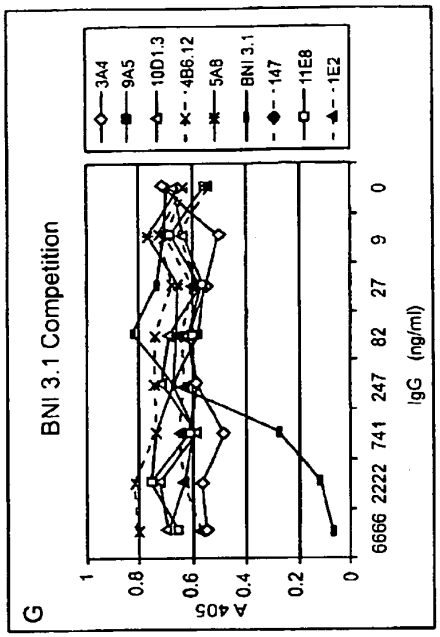


FIG. 13G

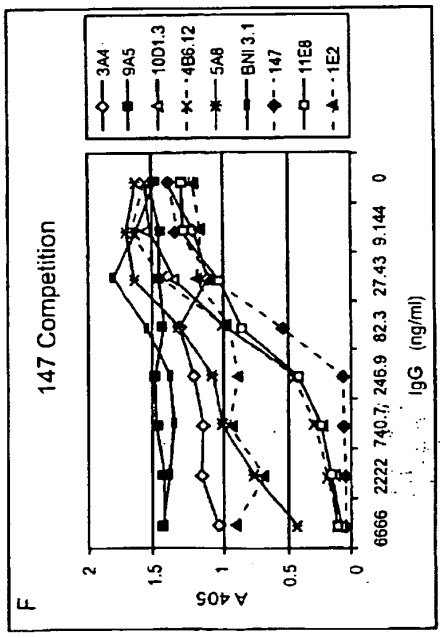


FIG. 13F

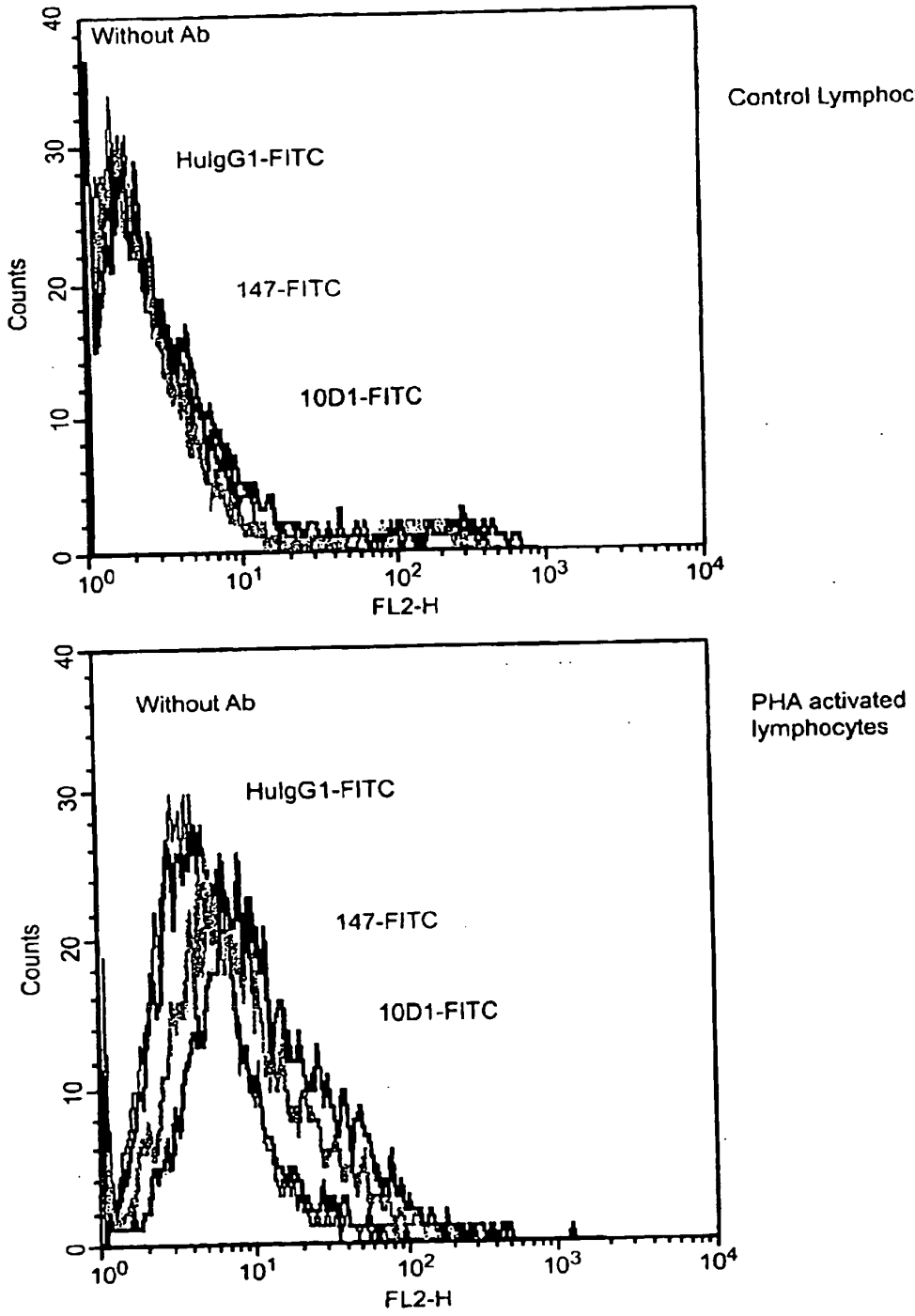

Without Ab
HulgG1-FITC
147-FITC
10D1-FITC
Counts
FL2-H
Control Lymphocytes
Without Ab
HulgG1-FITC
147-FITC
10D1-FITC
Counts
FL2-H
PHA activated lymphocytes

FIG. 14

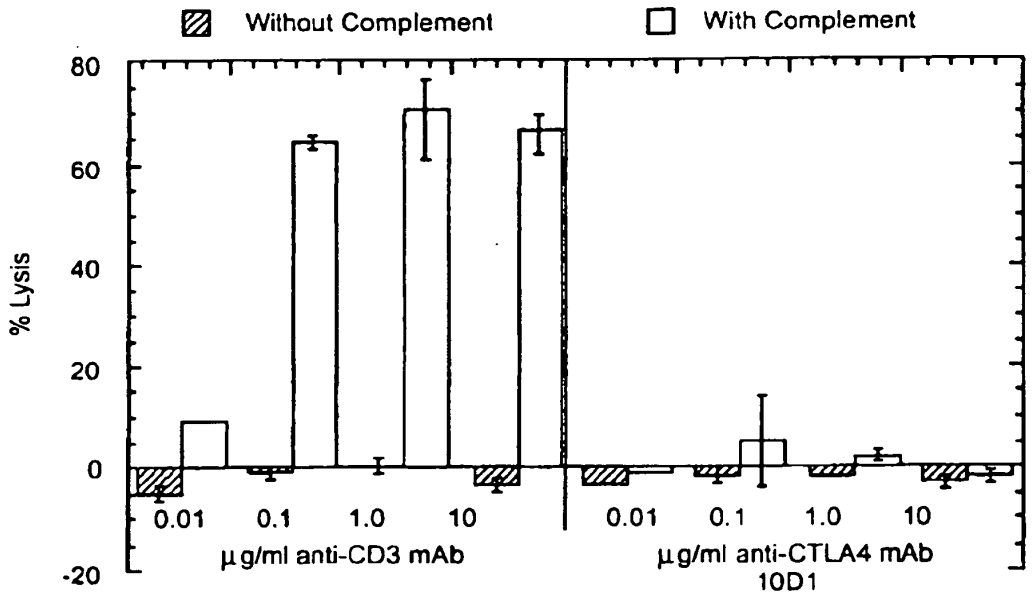

Without Complement
With Complement
80
60
40
20
0
-20
% Lysis
0.01
0.1
1.0
10
µg/ml anti-CD3 mAb
0.01
0.1
1.0
10
µg/ml anti-CTLA4 mAb
10D1

FIG. 15

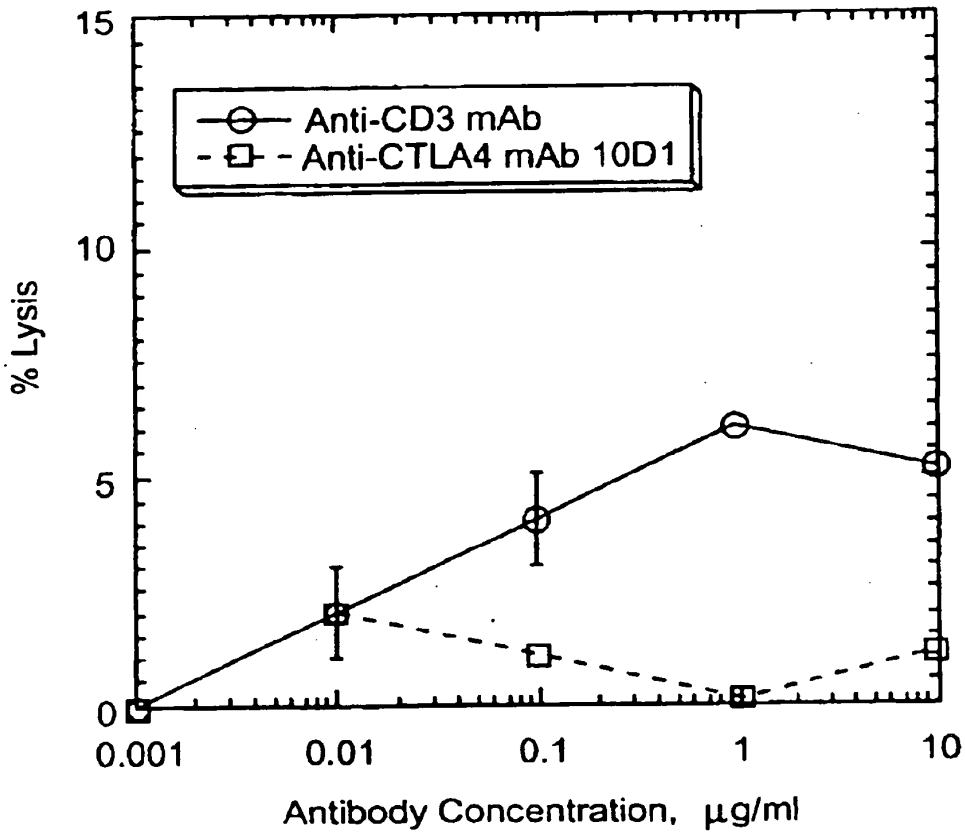

15
10
5
0
% Lysis
Anti-CD3 mAb
Anti-CTLA4 mAb 10D1
0.001
0.01
0.1
1
10
Antibody Concentration, µg/ml

FIG. 16

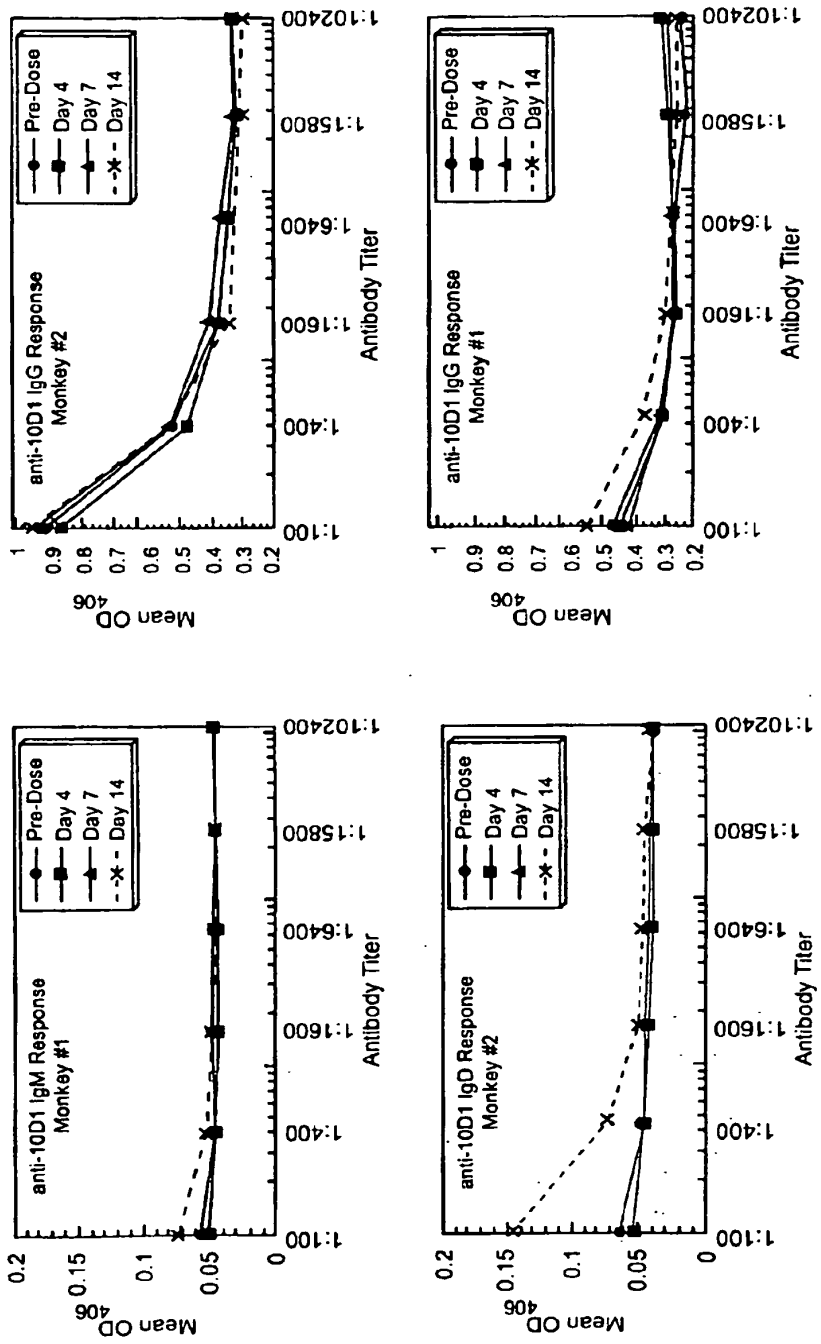

anti-10D1 IgG Response
Monkey #2
anti-10D1 IgG Response
Monkey #1
anti-10D1 IgM Response
Monkey #1
anti-10D1 IgD Response
Monkey #2
Pre-Dose
Day 4
Day 7
Day 14
Mean OD 406
Antibody Titer
1:100
1:400
1:1600
1:6400
1:15800
1:102400

FIG. 17

HUMAN CTLA-4 ANTIBODIES

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional patent application Ser. No. 60/150,452, filed Aug. 24, 1999 the disclosure of which is incorporated herein in its entirety.

FIELD OF THE INVENTION

The present invention relates generally to molecular immunology and the treatment of human diseases. In particular, it relates to novel human sequence antibodies against human CTLA-4 and methods of treating human diseases and infections using these antibodies.

BACKGROUND OF THE INVENTION

The vertebrate immune system requires multiple signals to achieve optimal immune activation; see, e.g., Janeway, Cold Spring Harbor Symp. *Quant. Biol.* 54:1–14 (1989); Paul William E., ed. Raven Press, N.Y., *Fundamental Immunology,* 4th edition (1998), particularly chapters 12 and 13, pages 411 to 478. Interactions between T lymphocytes (T cells) and antigen presenting cells (APC) are essential to the immune response. Levels of many cohesive molecules found on T cells and APC's increase during an immune response (Springer et al., *A. Rev. Immunol.* 5:223–252 (1987); Shaw and Shimuzu, *Current Opinion in Immunology,* Eds. Kindt and Long, 1:92–97 (1988)); and Hemler, *Immunology Today* 9:109–113 (1988)). Increased levels of these molecules may help explain why activated APC's are more effective at stimulating antigen-specific T cell proliferation than are resting APC's (Kaiuchi et al., *J. Immunol.* 131:109–114 (1983); Kreiger et al., *J. Immunol.* 135:2937–2945 (1985); McKenzie, J. Immunol. 141:2907–2911 (1988); and Hawrylowicz and Unanue, *J. Immunol.* 141:4083–4088 (1988)).

T cell immune response is a complex process that involves cell-cell interactions (Springer et al., *A. Rev. Immunol.* 5:223–252 (1987)), particularly between T and accessory cells such as APC's, and production of soluble immune mediators (cytokines or lymphokines) (Dinarello (1987) *New Engl. Jour. Med* 317:940–945; Sallusto (1997) *J. Exp. Med.* 179:1109–1118). This response is regulated by several T-cell surface receptors, including the T-cell receptor complex (Weiss (1986) *Ann. Rev. Immunol.* 4:593–619) and other "accessory" surface molecules (Allison (1994) *Curr. Opin. Immunol.* 6:414–419; Springer (1987) supra). Many of these accessory molecules are naturally occurring cell surface differentiation (CD) antigens defined by the reactivity of monoclonal antibodies on the surface of cells (McMichael, Ed., *Leukocyte Typing III,* Oxford Univ. Press, Oxford, N.Y. (1987)).

Early studies suggested that B lymphocyte activation requires two signals (Bretscher (1970) *Science* 169:1042–1049) and now it is believed that all lymphocytes require two signals for their optimal activation, an antigen specific or clonal signal, as well as a second, antigen non-specific signal. (Janeway, supra). Freeman (1989) *J. Immunol.* 143:2714–2722) isolated and sequenced a cDNA clone encoding a B cell activation antigen recognized by MAb B7 (Freeman (1987) *J. Immunol.* 138:3260). COS cells transfected with this cDNA have been shown to stain by both labeled MAb B7 and MAb BB-1 (Clark (1986) Human Immunol. 16:100–113; Yokochi (1981) *J. Immunol.* 128:823; Freeman et al., (1989) supra; Freeman et al. (1987), supra). In addition, expression of this antigen has been detected on cells of other lineages, such as monocytes (Freeman et al., supra).

T helper cell (Th) antigenic response requires signals provided by APC's. The first signal is initiated by interaction of the T cell receptor complex (Weiss, *J. Clin. Invest.* 86:1015 (1990)) with antigen presented in the context of class II major histocompatibility complex (MHC) molecules on the APC (Allen, *Immunol. Today* 8:270 (1987)). This antigen-specific signal is not sufficient to generate a fill response, and in the absence of a second signal may actually lead to clonal inactivation or anergy (Schwartz, *Science* 248:1349 (1990)). The requirement for a second "costimulatory" signal provided by the MHC has been demonstrated in a number of experimental systems (Schwartz, supra; Weaver and Unanue, *Immunol. Today* 11:49 (1990)). The molecular nature of this second signal is not completely understood, although it is clear in some cases that both soluble molecules such as interleukin (IL)-1 (Weaver and Unanue, supra) and membrane receptors involved in intercellular adhesion (Springer, *Nature* 346:425 (1990)) can provide costimulatory signals.

CD28 antigen, a homodimeric glycoprotein of the immunoglobulin superfamily (Aruffo and Seed, *Proc. Natl. Acad. Sci.* 84:8573–8577 (1987)), is an accessory molecule found on most mature human T cells (Damle et al., *J. Immunol.* 131:2296–2300 (1983)). Current evidence suggests that this molecule functions in an alternative T cell activation pathway distinct from that initiated by the T-cell receptor complex (June et al., *Mol. Cell. Biol.* 7:4472–4481 (1987)). Monoclonal antibodies (MAbs) reactive with CD28 antigen can augment T cell responses initiated by various polyclonal stimuli (reviewed by June et at., supra). These stimulatory effects may result from MAb-induced cytokine production (Thompson et al., *Proc. Natl. Acad. Sci* 86:1333–1337 (1989); and Lindsten et al., *Science* 244:339–343 (1989)) as a consequence of increased mRNA stabilization (Lindsten et al. (1989), supra). Anti-CD28 mAbs can also have inhibitory effects, i.e., they can block autologous mixed lymphocyte reactions (Damle et al., *Proc. Natl. Acad. Sci.* 78:5096–6001 (1981)) and activation of antigen-specific T cell clones (Lesslauer et al., *Eur. J. Immunol.* 16:1289–1296 (1986)).

Some studies have indicated that CD28 is a counter-receptor for the B cell activation antigen, B7/BB-1 (Linsley et al., *Proc. Natl. Acad. Sci. USA* 87:5031–5035 (1990)). The B7/BB-1 antigen is hereafter referred to as the "B7 antigen". The B7 ligands are also members of the immunoglobulin superfamily but have, in contrast to CD28, two Ig domains in their extracellular region, an N-terminal variable (V)-like domain followed by a constant (C)-like domain.

Delivery of a non-specific costimulatory signal to the T cell requires at least two homologous B7 family members found on APC's, B7-1 (also called B7, B7.1, or CD80) and B7-2 (also called B7.2 or CD86), both of which can deliver costimulatory signals to T cells via CD28. Costimulation through CD28 promotes T cell activation.

Using genetic fusions of the extracellular portions of B7 antigen and CD28 receptor, and Immunoglobulin (Ig) C.gamma.1 (constant region heavy chains), interactions between CD28 and B7 antigen have been characterized (Linsley et al., *J. Exp. Med.* 173:721–730 (1991)). Immobilized B7Ig fusion protein, as well as B7 positive CHO cells, have been shown to costimulate T cell proliferation.

T cell stimulation with B7 positive CHO cells also specifically stimulates increased levels of transcripts for IL-2. Additional studies have shown that anti-CD28 MAb inhibited IL-2 production induced in certain T cell leukemia cell

lines by cellular interactions with a B cell leukemia line (Kohno et al., Cell. Immunol. 131–1–10 (1990)).

CD28 has a single extracellular variable region (V)-like domain (Aruffo and Seed, supra). A homologous molecule, CTLA-4 has been identified by differential screening of a murine cytolytic-T cell cDNA library (Brunet (1987) Nature 328:267–270).

CTLA-4 is a T cell surface molecule that was originally identified by differential screening of a murine cytolytic T cell cDNA library (Brunet et al., *Nature* 328:267–270 (1987)). CTLA-4 is also a member of the immunoglobulin (Ig) superfamily; CTLA-4 comprises a single extracellular Ig domain. CTLA-4 transcripts have been found in T cell populations having cytotoxic activity, suggesting that CTLA-4 might function in the cytolytic response (Brunet et al., supra; Brunet et al., Immunol. Rev. 103-(21–36 (1988)). Researchers have reported the cloning and mapping of a gene for the human counterpart of CTLA-4 (Dariavach et al., *Eur. J. Immunol.* 18:1901–1905 (1988)) to the same chromosomal region (2q33–34) as CD28 (Lafage-Pochitaloff et al., *Immunogenetics* 31:198–201 (1990)). Sequence comparison between this human CTLA-4 DNA and that encoding CD28 proteins reveals significant homology of sequence, with the greatest degree of homology in the juxtamembrane and cytoplasmic regions (Brunet et al., 1988, supra; Dariavach et al., 1988, supra).

Some studies have suggested that CTLA-4 has an analogous function as a secondary costimulator (Linsley et al., *J Exp. Med.* 176:1595–1604 (1992); Wu et al., *J. Exp. Med.* 185:1327–1335 (1997) Lindsley, P. et al. U.S. Pat. Nos. 5,977,318; 5,968,510; 5,885,796; and 5,885,579). However, others have reported that CTLA-4 has an opposing role as a dampener of T cell activation (Krummel (1995) *J. Exp. Med.* 182:459–465); Krummel et al., *Int'l Immunol.* 8:519–523 (1996); Chambers et al., *Immunity.* 7:8855–895(1997)). It has been reported that CTLA-4 deficient mice suffer from massive lymphoproliferation (Chambers et al., supra). It has been reported that CTLA-4 blockade augments T cell responses in vitro (Walunas et al., *Immunity.* 1:405–413 (1994)) and in vivo (Kearney (1995) *J. Immunol.* 155:1032–1036), exacerbates antitumor immunity (Leach (1996) *Science.* 271:1734–1736), and enhances an induced autoimmune disease (Luhder (1998) *J Exp. Med.* 187:427–432). It has also been reported that CTLA-4 has an alternative or additional impact on the initial character of the T cell immune response (Chambers (1997) *Curr. Opin. Immunol.* 9:396404; Bluestone (1997) *J. Immunol.* 158:1989–1993; Thompson (1997) *Immunity* 7:445–450). This is consistent with the observation that some autoimmune patients have autoantibodies to CTLA-4. It is possible that CTLA-4 blocking antibodies have a pathogenic role in these patients (Matsui (1999) J. Immunol. 162:4328–4335).

Non-human CTLA-4 antibodies have be used in the various studies discussed above. However, one of the major impediments facing the development of in vivo therapeutic and diagnostic applications for antibodies in humans is the intrinsic immunogenicity of non-human immunoglobulins. For example, when immunocompetent human patients are administered therapeutic doses of rodent monoclonal antibodies, the patients produce antibodies against the rodent immunoglobulin sequences; these human anti-mouse antibodies (HAMA) neutralize the therapeutic antibodies and can cause acute toxicity. These and other deficiencies in the previous antibodies are overcome by the provision of human antibodies to CTLA-4 by the present invention.

SUMMARY OF THE INVENTION

The present invention provides a human sequence antibody that specifically binds to human CTLA-4 and a human sequence antibody that specifically binds to human CTLA-4 which is substantially free of non-immunoglobulin associated human proteins.

In a related aspect, the invention also provides a therapeutically-effective human sequence antibody that specifically binds to human CTLA-4. In some embodiments, the therapeutically-effective human sequence antibody binds to CTLA-4 on the cell surface of normal human T cells. In other embodiments, the T cell subpopulations marked by CD antigens CD4, CD8, CD25, and CD69 remain stable during and subsequent to the administration of the therapeutically-effective human sequence antibody. In other embodiments, the therapeutically-effective human sequence antibody binds CTLA-4 on the cell surface of normal human T cells. In other embodiments, the human sequence antibody is well-tolerated in a patient.

Also provided is a composition of polyclonal antibodies comprising a plurality of human sequence antibodies that specifically bind to human CTLA-4. The composition of polyclonal antibodies can comprise at least about 2, 5, 10, 50, 100, 500 or 1000 different human sequence antibodies that specifically bind to human CTLA-4.

The invention also provides human sequence antibodies that specifically bind to human CTLA-4 and which block binding of human CTLA-4 to human B7 or do not block binding of human CTLA-4 to human B7.

The invention also provides human sequence antibodies that bind to human CTLA-4 with an equilibrium association constant (Ka) of at least 10^8 M^{-1}. Also provided are human sequence antibodies that bind to human CTLA-4 with an equilibrium association constant (Ka) of at least 10^9 M^{-1}.

The invention also provides human sequence antibodies that specifically bind to human CTLA-4 that block binding of human CTLA-4 to human B7 by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%.

The invention also provides human sequence antibodies that specifically bind to human CTLA-4 having an antibody heavy chain of either IgG or IgM. The IgG antibody heavy chain can be IgG1, IgG2, IgG3 or IgG4. The invention also provides human sequence antibodies wherein the antibody light chain is a kappa light chain. The human sequence antibody can be encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:2 through SEQ ID NO:23, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:16 and SEQ ID NO:6, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:18 and SEQ ID NO:8, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:22 and SEQ ID NO:12, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by heavy

chain and light chain variable region amino acid sequences as set for the in SEQ ID NO:17 and SEQ ID NO:7, respectively.

The invention provides a human sequence antibody wherein the human sequence antibody is encoded by heavy chain and light chain variable region amino acid sequences as set for the in SEQ ID NO:19 and SEQ ID NO:9, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by heavy chain and light chain variable region amino acid sequences as set for the in SEQ ID NO:23 and SEQ ID NO:13, respectively.

The invention provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising variable heavy and light chain sequences from V gene segments VH 3-30.3 and VK A-27, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising variable heavy and light chain sequences from V gene segments VH 3-33 and VK L-15, respectively.

Some human sequence antibodies of the invention comprise heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGNNKYYADSVKG (SEQ ID NO:32) and TGWLGPFDY (SEQ ID NO:37), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVGSSYLA (SEQ ID NO:24), GAFSRAT (SEQ ID NO:29), and QQYGSSPWT (SEQ ID NO:35), respectively.

Some human sequence antibodies of the invention comprise heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGSNKHYADSVKG (SEQ ID NO:33) and TGWLGPFDY (SEQ ID NO: 37), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVSSSFLA (SEQ ID NO:25), GASSRAT (SEQ ID NO:30), and QQYGSSPWT (SEQ ID NO:35), respectively.

Other human sequence antibodies of the invention comprise heavy chain CDR1, CDR2, and CDR3 sequences, SYGMH (SEQ ID NO:28), VIWYDGSNKYYADSVKG (SEQ ID NO:34) and APNYIGAFDV (SEQ ID NO:38), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQGISSWLA (SEQ ID NO:26), AASSLQS (SEQ ID NO:31), and QQYNSYPPT (SEQ ID NO:36), respectively.

The invention also provides human sequence antibodies that specifically bind to human CTLA-4, wherein said human sequence antibody is produced by a transgenic non-human animal. The transgenic non-human animal can be a mouse.

The invention also provides a human sequence antibody that specifically bind to human CTLA-4 that is a Fab fragment.

The invention provides a polyvalent complex comprising at least two human sequence antibodies each of which specifically binds to human CTLA-4. The two different antibodies can be linked to each other covalently or non-covalently.

The invention provides a nucleic acid encoding a heavy chain of a human sequence antibody. The nucleic acid can comprise a nucleotide sequence as set forth in SEQ ID NO:1.

The invention provides a transgenic non-human animal having a genome comprising a human sequence heavy chain transgene and a human sequence light chain transgene, which animal has been immunized with a human CTLA-4, or a fragment or an analog thereof, whereby the animal expresses human sequence antibodies to the human CTLA-4. The transgenic non-human animal can be a transgenic mouse. The transgenic mouse can comprise HCo7 or HCo12.

The invention provides a hybridoma cell line comprising a B cell obtained from a transgenic non-human animal having a genome comprising a human sequence heavy chain transgene and a human sequence light chain transgene, wherein the hybridoma produces a human sequence antibody that specifically binds to human CTLA-4. In a related embodiment, the hybridoma secretes a human sequence antibody that specifically binds human CTLA-4 or binding fragment thereof, wherein the antibody is selected from the group consisting of: a human sequence antibody comprising heavy chain heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGNNKYYADSVKG (SEQ ID NO:32) and TGWLGPFDY (SEQ ID NO:37), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVGSSYLA (SEQ ID NO:24), GAFSRAT (SEQ ID NO:29), and QQYGSSPWT (SEQ ID NO:35), respectively, and heavy chain and light chain variable region amino acid sequences as set forth in SEQ ID NO:17 and SEQ ID NO:7, respectively; a human sequence antibody comprising heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGSNKHYADSVKG (SEQ ID NO:33) and TGWLGPFDY (SEQ ID NO: 37), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVSSSFLA (SEQ ID NO:25), GASSRAT (SEQ ID NO:30), and QQYGSSPWT (SEQ ID NO:35), respectively, and heavy chain and light chain variable region amino acid sequences as set forth in SEQ ID NO:19 and SEQ ID NO:9, respectively; or a human sequence antibody of claim 1, comprising heavy chain CDR1, CDR2, and CDR3 sequences, SYGMH (SEQ ID NO:28), VIWYDGSNKYYADSVKG (SEQ ID NO:34) and APNYIGAFDV (SEQ ID NO:38), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQGISSWLA (SEQ ID NO:26), AASSLQS (SEQ ID NO:31), and QQYNSYPPT (SEQ ID NO:36), respectively, and heavy chain and light chain variable region amino acid sequences as set forth in SEQ ID NO:23 and SEQ ID NO:13, respectively.

The invention provides a pharmaceutical composition comprising a human sequence antibody that specifically binds to human CTLA-4 and a pharmaceutically acceptable carrier. The pharmaceutical composition can further comprise an agent effective to induce an immune response against a target antigen. Also provided are chemotherapeutic agents. In addition, antibodies to immunosuppressive molecules are also provided.

The invention provides a method for inducing, augmenting or prolonging an immune response to an antigen in a patient, comprising administering to the patient an effective dosage of a human sequence antibody that specifically binds to human CTLA-4, wherein the antibody blocks binding of human CTLA-4 to human B7. The antigen can be a tumor antigen, or the antigen can be from a pathogen. The tumor antigen can also be telomerase. The pathogen can be a virus, a bacterium, a fungus or a parasite. The pathogen can also be an HIV. This method can further comprise administering the antigen, or a fragment or an analog thereof, to the patient, whereby the antigen in combination with the human sequence antibody induces, augments or prolongs the immune response. The antigen can be a tumor antigen or a

component of an amyloid formation in the patient, such as a patient suffering from Alzheimer's disease and the antigen is AB peptide. This method can further comprise administering a cytokine to the patient.

The invention provides a method of suppressing an immune response in a patient, comprising administering to the patient an effective dosage of a polyvalent preparation comprising at least two human sequence antibodies to human CTLA-4 linked to each other. The invention also provides a method of suppressing an immune response in a patient, comprising administering to the patient an effective dosage of a polyclonal preparation comprising at least two human sequence antibodies to human CTLA-4.

The present invention further provides isolated or recombinant human sequence antibodies and human monoclonal antibodies which specifically bind to human CTLA-4, as well as compositions containing one or a combination of such antibodies. Some of the human sequence antibodies of the invention are characterized by binding to human CTLA-4 with high affinity, and/or by blocking the interaction of human CTLA-4 with its ligand, the human B7-(1 and B7-(2 molecules. Accordingly, the human sequence antibodies and the human monoclonal antibodies of the invention can be used as diagnostic or therapeutic agents in vivo and in vitro.

The human sequence antibodies of the invention can encompass various antibody isotypes, or mixtures thereof, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE. Typically, they include IgG1 (e.g., IgG1k) and IgM isotypes. The human sequence antibodies can be full-length (e.g., an IgG1 or IgG4 antibody) or can include only an antigen-binding portion (e.g., a Fab, F(ab')2, Fv or a single chain Fv fragment). Some human sequence antibodies are recombinant human sequence antibodies. Some human sequence antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, e.g., a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene. The hybridoma can be made by, e.g., fusing the B cell to an immortalized cell. Some human sequence antibodies of the invention are produced by hybridomas referred to as 4C8, 4E10, 4E10.5, 5A8, 5C4, 5C4.1.3, 5D7, 5D7.1, 5E10, 5E10.12, 5G1, 5G1.4, 6A10, 6C9, 6C9.6, 6D9, 6D9.7, 6G4, 7E4, 7E4.4, 7E6, 7H8, 8E8, 8E8.4, 8F8, 8F8.19, 8H1, 9810, 9A10.1, 9B9, 9C1, 9G5, 105B, 10B5.8, 10B9, 10B9.2, 10D1, 10D1.3, 10E11, 10E4, 11E4.5, 11B4, 11D10, 11E4, 11E4.1, 11E8, 11F10, 11F11, 11F9, 11G1, 11G1.5, 1C7, 1H8.8, 2A7, 2A7.6, 2E2, 2E2.7, 2E7, 2E7.2, 2G1, 2G1.2, 3C12, 3E10, 3E10.5, 3E6, 3E6.0, 3F10, 4A1, 4B6 and 4B6.12. Suffixes after the decimal point indicate different clonal isolates of the same hybridoma cell lines.

Some human sequence anti-CTLA-4 antibodies of the present invention can be characterized by one or more of the following properties: a) specificity for human CTLA-4 (specifically binding to human CTLA-4); b) a binding affinity to human CTLA-4 with an equilibrium association constant (K_a) of at least about 10^7 M^{-1}, or about 10^9 M^{-1}, or about 10^{10} M^{-1} to 10^{11} M^{-1} or higher; c) a kinetic association constant (k_a) of at least about 10^3 about 10^4, or about 10^5 $m^{-1}s^{-1}$; and/or, d) a kinetic disassociation constant (k_d) of at least about 10^3, about 10^4, or about 10^5 $m^{-1}s^{-1}$.

In another aspect, the invention provides nucleic acid molecules encoding the human sequence antibodies, or antigen-binding portions, of the invention. Accordingly, recombinant expression vectors that include the antibody-encoding nucleic acids of the invention, and host cells transfected with such vectors, are also encompassed by the invention, as are methods of making the antibodies of the invention by culturing these host cells.

In yet another aspect, the invention provides isolated B-cells from a transgenic non-human animal, e.g., a transgenic mouse, which are capable of expressing various isotypes (e.g., IgG, IgA and/or IgM) of human monoclonal antibodies that specifically bind to human CTLA-4. The isolated B cells can be obtained from a transgenic non-human animal, e.g., a transgenic mouse, which has been immunized with a purified or enriched preparation of human CTLA-4 antigen (or antigenic fragment thereof) and/or cells expressing human CTLA-4. The transgenic non-human animal, e.g., a transgenic mouse, can have a genome comprising a human heavy chain transgene and a human light chain transgene. The isolated B-cells can be immortalized to provide a source (e.g., a hybridoma) of human monoclonal antibodies to human CTLA-4.

Accordingly, the present invention also provides a hybridoma capable of producing human monoclonal antibodies that specifically bind to human CTLA-4. The hybridoma can include a B cell obtained from a transgenic non-human animal, e.g., a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene fused to an immortalized cell. The transgenic non-human animal can be immunized with a purified or enriched preparation of human CTLA-4 antigen and/or cells expressing human CTLA-4 to generate antibody-producing hybridomas.

In yet another aspect, the invention provides a transgenic non-human animal, such as a transgenic mouse, which express human monoclonal antibodies (also referred to herein as a "HuMAb-Mouse™") that specifically bind to human CTLA-4. The transgenic non-human animal can be a transgenic mouse having a genome comprising a human heavy chain transgene and a human light chain transgene. The transgenic non-human animal can be immunized with a purified or enriched preparation of CTLA-4 antigen (or antigenic fragment thereof) and/or cells expressing the human CTLA-4. The transgenic non-human animal, e.g., the transgenic mouse, can be capable of producing multiple isotypes of human monoclonal antibodies to human CTLA-4 (e.g., IgG, IgA and/or IgM) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, e.g., classical or non-classical isotype switching.

In another aspect, the present invention provides methods for producing human sequence antibodies and human sequence monoclonal antibodies that specifically react with human CTLA-4. Some methods of the invention include immunizing a transgenic non-human animal, e.g., a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene, with a purified or enriched preparation of human CTLA-4 antigen and/or cells expressing human CTLA-4. B cells (e.g., splenic B cells) of the animal can then be obtained and fused with myeloma cells to form immortal, hybridoma cells that secrete human monoclonal antibodies against human CTLA-4.

Anti-human CTLA-4 human monoclonal antibodies of the invention, or antigen binding portions thereof (e.g., Fab), can be derivatized or linked to another functional molecule, e.g., another peptide or protein (e.g., an Fab' fragment). For example, an antibody or antigen-binding portion of the invention can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities. For

example, the human sequence anti-CTLA-4 antibody, or antigen binding fragment thereof, can be conjugated to a therapeutic moiety, e.g., a cytotoxic drug, an enzymatically active toxin, or a fragment thereof, a radioisotope, or a small molecule anti-cancer drug. The antibodies of the invention can also be conjugated to cytotoxic pharmaceuticals, e.g., radiolabeled with a cytotoxic agents, such as, e.g., ^{131}I(e.g., Shen (1997) Cancer 80(12 Suppl): 2553–2557), copper-67 (e.g., Deshpande (1988) J. Nucl. Med. 29:217–225) or, e.g., conjugation to the ribosome inactivating protein gelonin (e.g., Boyle (1996) J. of Immunol. 18:221–230).

In another aspect, the present invention provides compositions, e.g., pharmaceutical and diagnostic compositions, comprising a pharmaceutically acceptable carrier and at least one human monoclonal antibody of the invention, or an antigen-binding portion thereof, which specifically binds to human CTLA-4. Some compositions comprise a combination of the human sequence antibodies or antigen-binding portions thereof, preferably each of which binds to a distinct epitope. Compositions, e.g., pharmaceutical compositions, comprising a combination of at least one human sequence antibodies or at least one human monoclonal antibody of the invention, or antigen-binding portions thereof, and at least one bispecific or multispecific molecule of the invention, are also within the scope of the invention.

For in vivo methods, the antibody, or antigen-binding portion thereof (or a bispecific or multispecific molecule of the invention), can be administered to a human subject suffering from a T-cell-related disease, or a disease that can be ameliorated or prevented by augmenting or suppressing or prolonging an immune response.

Human sequence monoclonal antibody and human sequence antibody compositions of the invention also can be administered in combination with other known therapies, e.g., an anti-cancer therapy. Accordingly, the invention provides a method for treating cancer in a subject comprising administering a therapeutically effective amount of a pharmaceutical composition of a human sequence antibody together with a pharmaceutical carrier to the subject. Some such methods include a vaccine. Some such vaccines include a tumor cell vaccine, a GM-CSF-modified tumor cell vaccine, or an antigen-loaded dendritic cell vaccine. In some such methods, the cancer is prostate cancer, melanoma, or epithelial cancer.

Human sequence antibodies to human CTLA-4 can be used in methods of treatment requiring either stimulation of immune responses or suppression. The former indication is treated using antibodies that block binding of human CTLA-4 to human B7. Diseases amenable to treatment by stimulation, augmentation of prolonging of immune responses including cancer, including cancers of the prostate, kidney or colon, pathogenic infections, diseases associated with auto-antigens, e.g., amyloidogenic diseases, including Alzheimer's disease, and diseases with inflammatory or allergic components. Immunosuppression is achieved using a polyvalent preparation comprising at least two different antibodies to human CTLA-4 that are linked to each other. Diseases amenable to treatment include graft versus host disease, host versus graft disease, autoimmune diseases and inflammation.

In yet another aspect, the present invention provides a method for detecting in vitro or in vivo the presence of human CTLA-4 antigen in a-sample, e.g., for diagnosing a human CTLA-4-related disease. In some methods, this is achieved by contacting a sample to be tested, along with a control sample, with a human sequence antibody or a human monoclonal antibody of the invention, or an antigen-binding portion thereof (or a bispecific or multispecific molecule), under conditions that allow for formation of a complex between the antibody and human CTLA-4. Complex formation is then detected (e.g., using an ELISA) in both samples, and any statistically significant difference in the formation of complexes between the samples is indicative the presence of human CTLA-4 antigen in the test sample.

A further understanding of the nature and advantages of the present invention may be realized by reference to the remaining portions of the specification, the figures and claims.

All publications, figures, GenBank Accession references (sequences), ATCC Deposits, patents and patent applications cited herein are hereby expressly incorporated by reference for all purposes to the same extent as if each was so individually denoted.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows schematics illustrating the targeted insertion of a neo cassette into the Sma I site of the μ1 exon. FIG. 1A) Schematic diagram of the genomic structure of the μ locus. The filled boxes represent the μ exons; FIG. 1B) Schematic diagram of the CmD targeting vector. The dotted lines denotes those genomic μ sequences included in the construct. Plasmid sequences are not shown; FIG. 1C) Schematic diagram of the targeted μ locus in which the neo cassette has been inserted into μl. The box at the lower right shows those RFLP's diagnostic of homologous recombination between the targeting construct and the μ locus. The RFLP's were detected by Southern blot hybridization using probe A, the 915 bp Sac I fragment is shown in FIG. 1C.

FIG. 2 shows the results of experiments demonstrating that soluble human sequence antibodies against human CTLA-4 inhibit the binding of recombinant soluble human CTLA-4 to cells expressing mouse B7.1, as described in detail, below.

FIG. 3 shows the results of a competitive binding assay to identify human sequence antibodies of the invention that recognize non-overlapping epitopes on human CTLA-4, as described in detail, below.

FIG. 4 shows preliminary nucleotide sequence data for the heavy and light chain fragment of the anti-CTLA-4 antibody 10D1.3.

FIG. 5 shows the nucleotide sequences of the light chain variable Regions (V_K) of Anti-Human CTLA-4 Antibodies. The anti-CTLA-4 antibodies 10D1 (SEQ ID NO:6) and 4B6 (SEQ ID NO:8) derived from the V_K A-27 germline sequence (SEQ ID NO:4) are depicted at the top of the Figure. The anti-CTLA-4 antibody 1E2 (SEQ ID NO:12) derived from the V_K L-15 germline sequence (SEQ ID NO:10) is shown at the bottom of the Figure. The V_K sequences of three anti-CTLA-4 antibodies are aligned with their germline encoded VK gene sequences. The complementary determining residues (CDR) are labeled. Dashes denote sequence identity.

FIG. 6 shows the nucleotide sequences of the heavy chain variable Regions (V_H) of Anti-Human CTLA-4 Antibodies. The anti-CTLA-4 antibodies 10D1 (SEQ ID NO:16) and 4B6 (SEQ ID NO:18) derived from the V_H 3-30.3 germline sequence (SEQ ID NO:14) are depicted at the top of the Figure. The anti-CTLA-4 antibody 1E2 (SEQ ID NO:22) derived from the V_H 3–33 germline sequence (SEQ ID NO:20) is shown at the bottom of the Figure. The V_H sequences of three anti-CTLA-4 antibodies are aligned with

their germline encoded sequences. The complementary determining residues (CDR) are labeled. Dashes denote sequence identity.

FIG. **7** shows the predicted amino acid sequences of the light chain Variable Regions of Anti-Human CTLA-4 Antibodies. The predicted amino acid V_K sequences of the anti-CTLA-4 antibodies described in FIG. **5** are shown. The anti-CTLA-4 antibodies 10D1 (SEQ ID NO:7) and 4B6 (SEQ ID NO:9) derived from the V_K A-27 germline sequence (SEQ ID NO:5) are depicted at the top of the Figure. The anti-CTLA-4 antibody 1E2 (SEQ ID NO:13) derived from the V_K L-15 germline sequence (SEQ ID NO:11) is shown at the bottom of the Figure.

FIG. **8** shows the predicted amino acid sequences of the heavy chain Variable Regions of Anti-Human CTLA-4 Antibodies. The predicted amino acid V_H sequences of the anti-CTLA-4 antibodies described in FIG. **6** are shown. The anti-CTLA-4 antibodies 10D1 (SEQ ID NO:17) and 4B6 (SEQ ID NO:19) derived from the V_H, 3-30.3 germline sequence (SEQ ID NO:15) are depicted at the top of the Figure. The anti-CTLA-4 antibody 1E2 (SEQ ID NO:23) derived from the V_H 3-33 germline sequence (SEQ ID NO:21) is shown at the bottom of the Figure.

FIG. **9** shows the results of binding experiments of MAb 10D1 to recombinant human CTLA-4 by ELISA. MAb 10D1 binds with dose-dependent and saturating kinetics to purified recombinant CTLA-4.

FIG. **10** shows the binding of 10D1 to a CTLA-4 expressing T-cell line. These data show that MAb 10D1 binds with dose-dependent and saturating kinetics to cells expressing CTLA-4.

FIG. **11** shows inhibition of binding of human B7.2 Ig to CTLA-4-expressing T-cells. These data show that MAb 10D1 can efficiently block B7.2 binding to CTLA-4 as compared to a control human MAb.

FIG. **12** shows the results for blocking CTLA-4-FITC binding to murine B7.1-expressing cells. These data show that MAb 10D1 can efficiently block CTLA-4 binding to B7.1 as compared to a control human MAb.

FIG. **13** shows competitive ELISAs of anti-CTLA-4 human MAbs demonstrating epitope group classifications.

FIG. **14** shows CTLA-4 expression on PHA-stimulated T-cells. Activated, but not resting T cells, express low but detectable levels of CTLA-4 at the cell surface.

FIG. **15** shows the results of MAb 10D1 in Complement Dependent Lysis of Activated T Cells. No lysis of PHA-activated T cells is observed.

FIG. **16** shows the results of MAb 10D1 in Antibody-Dependent Lysis of Activated T Cells. No lysis of PHA-activated T cells is observed with 10D1 and mononuclear cells.

FIG. **17** shows anti-10D1 IgM and IgG responses in cynomolgus monkeys injected with 10D1 antibody. No significant antibody response to 10D1 is observed.

DETAILED DESCRIPTION

The present invention provides novel antibody-based therapies for treating and diagnosing diseases characterized by expression, particularly over-expression, or activation of, particularly overactivation, of human CTLA-4 and/or related molecules. Therapies of the invention employ human sequence antibodies, human sequence monoclonal antibodies, or antigen-binding portions thereof, which bind to an epitope present on human CTLA-4. These human sequence anti-CTLA-4 antibodies can act as functional antagonists (e.g., inhibiting the ability of CTLA-4 to bind ligand or to activate the cell, e.g., by inhibiting its ability to transmit a signal to the cell) or agonists (e.g., to simulate the effect of ligand).

The human sequence antibodies of the invention can be produced in a non-human transgenic animal, e.g., a transgenic mouse, capable of producing multiple isotypes of human (e.g., monoclonal or polyclonal) antibodies to human CTLA-4 (e.g., IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Accordingly, various aspects of the invention include antibodies and antibody fragments, and pharmaceutical compositions thereof, as well as non-human transgenic animals, and B-cells and hybridomas for making such monoclonal antibodies. Methods of using the antibodies of the invention to detect a cell expressing human CTLA-4 or a related, cross-reactive growth factor receptor, or to inhibit growth, differentiation and/or motility of a cell expressing human CTLA-4, either in vitro or in vivo, are also encompassed by the invention.

Except when noted, the terms "patient" or "subject" are used interchangeably and refer to mammals such as human patients and non-human primates, as well as experimental animals such as rabbits, rats, and mice, and other animals.

The term "treating" includes the administration of the compounds or agents of the present invention to prevent or delay the onset of the symptoms, complications, or biochemical indicia of a disease, alleviating the symptoms or arresting or inhibiting further development of the disease, condition, or disorder (e.g., autoimmune disease). Treatment may be prophylactic (to prevent or delay the onset of the disease, or to prevent the manifestation of clinical or subclinical symptoms thereof) or therapeutic suppression or alleviation of symptoms after the manifestation of the disease.

In general, the phrase "sell tolerated" refers to the absence of adverse changes in health status that occur as a result of the treatment and would affect treatment decisions.

The term "lymphocyte" as used herein has the normal meaning in the art, and refers to any of the mononuclear, nonphagocytic leukocytes, found in the blood, lymph, and lymphoid tissues, i.e., B and T lymphocytes.

The phrase "subpopulations of T lymphocytes" or "T cell subset(s)" refers to T lymphocytes or T cells characterized by the expression of particular cell surface markers (see Barclay, A. N. et al. (eds.), 1997, The Leukocyte Antigen Facts Book, 2nd. edition, Academic Press, London, United Kingdom). The term "stable" in reference to T cells refers to the fact that the frequency or percentage of a T cell subset does not change over the course or duration of the administration of an agent.

The terms "cytotoxic T lymphocyte-associated antigen-4," "CTLA-4," "CTLA-4," "CTLA-4 antigen" and "CD152" (see, e.g., Murata (1999) Am. J. Pathol. 155:453–460) are used interchangeably, and include variants, isoforms, species homologs of human CTLA-4, and analogs having at least one common epitope with CTLA-4 (see, e.g., Balzano (1992) Int. J. Cancer Suppl. 7:28–32).

The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. The region of amino acids 1–37 is the leader peptide; 38–161 is the extracellular V-like domain; 162–187 is the transmembrane domain; and 188–223 is the cytoplasmic domain. Variants of the nucleotide sequence have been reported, including a G to A transition at position **49**, a C to T transition at position **272**, and an A to G transition at position **439**. The complete DNA

sequence of mouse CTLA-4 has the EMBL accession number X05719 (Brunet et al., (1987) Nature 328:267–270). The region of amino acids 1–35 is the leader peptide.

The complete DNA sequence of human B7-1 (CD80) has the Genbank accession number X60958; the accession number for the mouse sequence is X60958; the accession number for the rat sequence is U05593. The complete cDNA sequence of human B7-2 (CD86) has the Genbank accession number L25259; the accession number for the mouse sequence is L25606.

The genes encoding CD28 have been extensively characterized. The chicken mRNA sequence has the Genbank accession number X67915. The rat mRNA sequence has the Genbank accession number X55288. The human mRNA sequence has the Genbank accession number J02988. The mouse mRNA sequence has the Genbank accession number M34536.

The term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

An intact "antibody" comprises at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The term antibody includes antigen-binding portions of an intact antibody that retain capacity to bind CTLA-4. Examples of binding include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) *Nature* 341:544–546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); See, e.g., Bird et al. (1988) *Science* 242:423–426; and Huston et al. (1988) *Proc. Natl. Acad. Sci.* USA 85:5879–5883). Such single chain antibodies are included by reference to the term "antibody" Fragments can be prepared by recombinant techniques or enzymatic or chemical cleavage of intact antibodies.

A bispecific antibody has two different binding specificities, see. e.g., U.S. Pat. Nos. 5,922,845 and 5,837,243; Zeilder (1999) J. Immunol. 163:1246–1252; Somasundaram (1999) Hum. Antibodies 9:47–54; Keler (1997) Cancer Res. 57:4008–401.4. For example, the invention provides bispecific antibodies having one binding site for a cell surface antigen, such as human CTLA-4, and a second binding site for an Fc receptor on the surface of an effector cell. The invention also provides multispecific antibodies, which have at least three binding sites. The term "bispecific antibodies" further includes diabodies. Diabodies are bivalent, bispecific antibodies in which the VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (See, e.g., Holliger, P., et al. (1993) *Proc. Natl. Acad. Sci.* USA 90:6444–6448; Poljak, R. J., et al. (1994) Structure 2:1121–1123).

The term "human sequence antibody" includes antibodies having variable and constant regions (if present) derived from human germline immunoglobulin sequences. The human sequence antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human sequence antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (i.e., humanized antibodies).

The terms "monoclonal antibody" or "monoclonal antibody composition" refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions (if present) derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, e.g., a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "diclonal antibody" refers to a preparation of at least two antibodies to human CTLA-4. Typically, the different antibodies bind different epitopes.

The term "oligoclonal antibody" refers to a preparation of 3 to 100 different antibodies to human CTLA-4. Typically, the antibodies in such a preparation bind to a range of different epitopes.

The term "polyclonal antibody" refers to a preparation of more than 1 (two or more) different antibodies to human CTLA-4. Such a preparation includes antibodies binding to a range of different epitopes.

The invention provides human sequence antibodies to human CTLA-4 which block or antagonize signals transduced by the human CTLA-4 receptor. Some of these antibodies can bind to an epitope on human CTLA-4 so as to inhibit CTLA-4 from interacting with a human B7 coun-

terreceptor. Because interaction of human CTLA-4 with human B7 transduces a signal leading to inactivation of T-cells bearing the human CTLA-4 receptor, antagonism of the interaction effectively induces, augments or prolongs the activation of T cells bearing the human CTLA-4 receptor, thereby prolonging or augmenting an immune response. A "blocking antibody" refers to an antibody that reduces the binding of soluble human CTLA-4 to cell-expressed human B7 ligand by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99% or 99.9% under conditions in which the ratio of antibody combining site to human CTLA-4 ligand binding site is greater than 1:1 and the concentration of antibody is greater than 10^{-8} M.

Other antibody preparations, sometimes referred to as multivalent preparations, bind to human CTLA-4 in such a manner as to crosslink multiple human CTLA-4 receptors on the same cell. Cross-linking of receptor has the same or similar effect to binding of human CTLA-4 to human B7. Thus, cross-linking of receptors effectively agonizes the human CTLA-4 response resulting in immunosuppression.

Cross-linking can also be accomplished by combining soluble divalent antibodies having different epitope specificities. These polyclonal antibody preparations comprise at least two pairs of heavy and light chains binding to different epitopes on human CTLA-4 such that an immunosuppressing signal can be transduced as a result of human CTLA-4 crosslinking.

The term "recombinant human antibody" includes all human sequence antibodies of the invention that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (described further in Section I, below); antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions (if present) derived from human germline immunoglobulin sequences. Such antibodies can, however, be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

A "heterologous antibody" is defined in relation to the transgenic non-human organism producing such an antibody. This term refers to an antibody having an amino acid sequence or an encoding nucleic acid sequence corresponding to that found in an organism not consisting of the transgenic non-human animal, and generally from a species other than that of the transgenic non-human animal.

A "heterohybrid antibody" refers to an antibody having a light and heavy chains of different organismal origins. For example, an antibody having a human heavy chain associated with a murine light chain is a heterohybrid antibody. Examples of heterohybrid antibodies include chimeric and humanized antibodies, discussed supra.

The term "substantially pure" or "isolated" means an object species (e.g., an antibody of the invention) has been identified and separated and/or recovered from a component of its natural environment such that the object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition); a "substantially pure" or "isolated" composition also means where the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. A substantially pure or isolated composition can also comprise more than about 80 to 90 percent by weight of all macromolecular species present in the composition. An isolated object species (e.g., antibodies of the invention) can also be purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of derivatives of a single macromolecular species. An isolated antibody to human CTLA-4 can be substantially free of other antibodies that lack binding to human CTLA-4 and bind to a different antigen. An isolated antibody that specifically binds to an epitope, isoform or variant of human CTLA-4 may, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., CTLA-4 species homologs). Moreover, an isolated antibody of the invention be substantially free of other cellular material (e.g., non-immunoglobulin associated proteins) and/or chemicals.

"Specific binding" refers to antibody binding to a predetermined antigen. The phrase "specifically (or selectively) binds" to an antibody refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Typically, the antibody binds with an association constant (K_a) of at least about 1×10^6 M^{-1} or 10^7 M^{-1}, or about 10^8 M^{-1} to 10^9 M^{-1}, or about 10^9 M^{-1} to 10^{11} M^{-1} or higher, and binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen".

The phrase "specifically bind(s)" or "bind(s) specifically" when referring to a peptide refers to a peptide molecule which has intermediate or high binding affinity, exclusively or predominately, to a target molecule. The phrases "specifically binds to" refers to a binding reaction which is determinative of the presence of a target protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated assay conditions, the specified binding moieties bind preferentially to a particular target protein and do not bind in a significant amount to other components present in a test sample. Specific binding to a target protein under such conditions may require a binding moiety that is selected for its specificity for a particular target antigen. A variety of assay formats may be used to select ligands that are specifically reactive with a particular protein. For example, solid-phase ELISA immunoassays, immunoprecipitation, Biacore and Western blot are used to identify peptides that specifically react with CTLA-4. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 times background.

The term "high affinity" for an IgG antibody refers to an equilibrium association constant (K_a) of at least about 10^7M^{-1}, at least about 10^8M^{-1}, at least about 10^9M^{-1}, at least about $10^{10}M^{-1}$, at least about $10^{11}M^{-1}$, or at least about $10^{12}M^{-1}$ or greater, e.g., up to $10^{13}M^{-1}$ or $10^{14}M^{-1}$ or greater. However, "high affinity" binding can vary for other antibody isotypes.

The term "K_a", as used herein, is intended to refer to the equilibrium association constant of a particular antibody-antigen interaction. This constant has units of 1/M.

The term "K_a", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction. This constant has units of M.

The term "k_a", as used herein, is intended to refer to the kinetic association constant of a particular antibody-antigen interaction. This constant has units of 1/Ms.

The term "k_d", as used herein, is intended to refer to the kinetic dissociation constant of a particular antibody-antigen interaction. This constant has units of 1/s.

"Particular antibody-antigen interactions" refers to the experimental conditions under which the equilibrium and kinetic constants are measured.

"Isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

"Isotype switching" refers to the phenomenon by which the class, or isotype, of an antibody changes from one Ig class to one of the other Ig classes.

"Nonswitched isotype" refers to the isotypic class of heavy chain that is produced when no isotype switching has taken place; the CH gene encoding the nonswitched isotype is typically the first CH gene immediately downstream from the functionally rearranged VDJ gene. Isotype switching has been classified as classical or non-classical isotype switching. Classical isotype switching occurs by recombination events which involve at least one switch sequence region in the transgene. Non-classical isotype switching may occur by, for example, homologous recombination between human σ_μ, and human Σ_μ (δ-associated deletion). Alternative non-classical switching mechanisms, such as intertransgene and/or interchromosomal recombination, among others, may occur and effectuate isotype switching.

The term "switch sequence" refers to those DNA sequences responsible for switch recombination. A "switch donor" sequence, typically a μ switch region, are 5' (i.e., upstream) of the construct region to be deleted during the switch recombination. The "switch acceptor" region are between the construct region to be deleted and the replacement constant region (e.g., γ, ϵ, etc.). As there is no specific site where recombination always occurs, the final gene sequence is not typically predictable from the construct.

"Glycosylation pattern" is defined as the pattern of carbohydrate units that are covalently attached to a protein, more specifically to an immunoglobulin protein. A glycosylation pattern of a heterologous antibody can be characterized as being substantially similar to glycosylation patterns which occur naturally on antibodies produced by the species of the non-human transgenic animal, when one of ordinary skill in the art would recognize the glycosylation pattern of the heterologous antibody as being more similar to said pattern of glycosylation in the species of the non-human transgenic animal than to the species from which the CH genes of the transgene were derived.

The term "naturally-occurring" as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "rearranged" refers to a configuration of a heavy chain or light chain immunoglobulin locus wherein a V segment is positioned immediately adjacent to a D-J or J segment in a conformation encoding essentially a complete VH or VL domain, respectively. A rearranged immunoglobulin gene locus can be identified by comparison to germline DNA; a rearranged locus has at least one recombined heptamer/nonamer homology element.

The term "unrearranged" or "germline configuration" in reference to a V segment refers to the configuration wherein the V segment is not recombined so as to be immediately adjacent to a D or J segment.

The term "nucleic acid" is intended to include DNA molecules and RNA molecules. A nucleic acid can be single-stranded or double-stranded.

The term "isolated nucleic acid" in reference to nucleic acids encoding antibodies or antibody portions (e.g., VH, VL, CDR3) that bind to CTLA-4, is intended to refer to a nucleic acid in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than CTLA-4, which other sequences may naturally flank the nucleic acid in human genomic DNA. SEQ ID NOs: 4–23 comprise the nucleotide and amino acid sequences comprising the heavy chain (VH) and light chain (VL) variable regions of the 10D1, 4B6 and 1E2 human anti-CTLA-4 monoclonal antibodies of the invention.

The term "substantially identical," in the context of two nucleic acids or polypeptides refers to two or more sequences or subsequences that have at least about 80%, about 90, about 95% or higher nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using the following sequence comparison method and/or by visual inspection. For example, the invention provides nucleic acids having sequences that are substantially identical to SEQ ID NO:1, SEQ ID NO:2. Such "substantially identical" sequences are typically considered to be homologous. The "substantial identity" can exist over a region of sequence that is at least about 50 residues in length, over a region of at least about 100 residues, or over a region at least about 150 residues, or over the full length of the two sequences to be compared. As described below, any two antibody sequences can only be aligned in one way, by using the numbering scheme in Kabat. Therefore, for antibodies, percent identity has a unique and well-defined meaning.

Amino acids from the variable regions of the mature heavy and light chains of immunoglobulins are designated Hx and Lx respectively, where x is a number designating the position of an amino acid according to the scheme of Kabat, *Sequences of Proteins of immunological Interest* (National Institutes of Health, Bethesda, Md., 1987 and 1991). Kabat lists many amino acid sequences for antibodies for each subgroup, and lists the most commonly occurring amino acid for each residue position in that subgroup to generate a consensus sequence. Kabat uses a method for assigning a residue number to each amino acid in a listed sequence, and this method for assigning residue numbers has become standard in the field. Kabat's scheme is extendible to other antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. The use of the Kabat numbering system readily identifies amino acids at equivalent positions in different antibodies. For example, an amino acid at the L50 position of a human antibody occupies the equivalent position to an amino acid position L50 of a mouse antibody. Likewise, nucleic acids encoding antibody chains are aligned when the amino acid sequences encoded by the respective nucleic acids are aligned according to the Kabat numbering convention.

The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a mol-

ecule to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA), wherein the particular nucleotide sequence is detected at least at about 10 times background. In one embodiment, a nucleic acid can be determined to be within the scope of the invention (e.g., is substantially identical to SEQ ID NO:1 or SEQ ID NO:2) by its ability to hybridize under stringent conditions to a nucleic acid otherwise determined to be within the scope of the invention (such as the exemplary sequences described herein).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but not to other sequences in significant amounts (a positive signal (e.g., identification of a nucleic acid of the invention) is about 10 times background hybridization). Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found An extensive guide to the hybridization of nucleic acids is found in e.g., Sambrook, ed., MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1–3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part 1. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

Generally, stringent conditions are selected to be about 5–10°C. lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C. for short probes (e.g., 10 to 50 nucleotides) and at least about 60° C. for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide as described in Sambrook (cited below). For high stringency hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary high stringency or stringent hybridization conditions include: 50% formamide, 5xSSC and 1% SDS incubated at 42° C. or 5xSSC and 1% SDS incubated at 65° C., with a wash in 0.2xSSC and 0.1% SDS at 65° C. For selective or specific hybridization, a positive signal (e.g., identification of a nucleic acid of the invention) is about 10 times background hybridization. Stringent hybridization conditions that are used to identify nucleic acids within the scope of the invention include, e.g., hybridization in a buffer comprising 50% formamide, 5xSSC, and 1% SDS at 42° C., or hybridization in a buffer comprising 5xSSC and 1% SDS at 65° C., both with a wash of 0.2xSSC and 0.1% SDS at 65° C. In the present invention, genomic DNA or cDNA comprising nucleic acids of the invention can be identified in standard Southern blots under stringent conditions using the nucleic acid sequences disclosed here. Additional stringent conditions for such hybridizations (to identify nucleic acids within the scope of the invention) are those which include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37° C.

However, the selection of a hybridization format is not critical—it is the stringency of the wash conditions that set forth the conditions which determine whether a nucleic acid is within the scope of the invention. Wash conditions used to identify nucleic acids within the scope of the invention include, e.g.: a salt concentration of about 0.02 molar at pH 7 and a temperature of at least about 50° C. or about 55° C. to about 60° C.; or, a salt concentration of about 0.15 M NaCl at 72° C. for about 15 minutes; or, a salt concentration of about 0.2xSSC at a temperature of at least about 50° C. or about 55° C. to about 60° C. for about 15 to about 20 minutes; or, the hybridization complex is washed twice with a solution with a salt concentration of about 2xSSC containing 0.1% SDS at room temperature for 15 minutes and then washed twice by 0.1xSSC containing 0.1% SDS at 68° C. for 15 minutes; or, equivalent conditions. See Sambrook, Tijssen and Ausubel for a description of SSC buffer and equivalent conditions.

The nucleic acids of the invention be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. see, e.g., Sambrook, Tijssen and Ausubel. The nucleic acid sequences of the invention and other nucleic acids used to practice this invention, whether RNA, cDNA, genomic DNA, or hybrids thereof, may be isolated from a variety of sources, genetically engineered, amplified, and/or expressed recombinantly. Any recombinant expression system can be used, including, in addition to bacterial, e.g., yeast, insect or mammalian systems. Alternatively, these nucleic acids can be chemically synthesized in vitro. Techniques for the manipulation of nucleic acids, such as, e.g., subcloning into expression vectors, labeling probes, sequencing, and hybridization are well described in the scientific and patent literature, see, e.g., Sambrook, Tijssen and Ausubel. Nucleic acids can be analyzed and quantified by any of a number of general means well known to those of skill in the art. These include, e.g., analytical biochemical methods such as NMR, spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and hyperdiffusion chromatography, various immunological methods, such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, Southern analysis, Northern analysis, dot-blot analysis, gel electrophoresis (e.g., SDS-PAGE), RT-PCR, quantitative PCR, other nucleic acid or target or signal amplification methods, radiolabeling, scintillation counting, and affinity chromatography.

The nucleic acid compositions of the present invention, while often in a native sequence (except for modified restriction sites and the like), from either cDNA, genomic or mixtures may be mutated, thereof in accordance with standard techniques to provide gene sequences. For coding sequences, these mutations, may affect amino acid sequence as desired. In particular, DNA sequences substantially homologous to or derived from native V, D, J, constant, switches and other such sequences described herein are contemplated (where "derived" indicates that a sequence is identical or modified from another sequence).

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence.

For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. With respect to transcription regulatory sequences, operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. For switch sequences, operably linked indicates that the sequences are capable of effecting switch recombination.

The term "vector" is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell") refers to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

The term "minilocus transgene" refers to a transgene that comprises a portion of the genomic immunoglobulin locus having at least one internal (i.e., not at a terminus of the portion) deletion of a non-essential DNA portion (e.g., intervening sequence; intron or portion thereof) as compared to the naturally-occurring germline Ig locus.

A "label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins for which antisera or monoclonal antibodies are available (e.g., the polypeptides of the invention can be made detectable, e.g., by incorporating a radiolabel into the peptide, and used to detect antibodies specifically reactive with the peptide).

The term "sorting" in the context of cells as used herein to refers to both physical sorting of the cells, as can be accomplished using, e.g., a fluorescence activated cell sorter, as well as to analysis of cells based on expression of cell surface markers, e.g., FACS analysis in the absence of sorting.

The phrase "immune cell response" refers to the response of immune system cells to external or internal stimuli (e.g., antigen, cytokines, chemokines, and other cells) producing biochemical changes in the immune cells that result in immune cell migration, killing of target cells, phagocytosis, production of antibodies, other soluble effectors of the immune response, and the like.

The terms "T lymphocyte response" and "T lymphocyte activity" are used here interchangeably to refer to the component of immune response dependent on T lymphocytes (i.e., the proliferation and/or differentiation of T lymphocytes into helper, cytotoxic killer, or suppressor T lymphocytes, the provision of signals by helper T lymphocytes to B lymphocytes that cause or prevent antibody production, the killing of specific target cells by cytotoxic T lymphocytes, and the release of soluble factors such as cytokines that modulate the function of other immune cells).

The term "immune response" refers to the concerted action of lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

Components of an immune response may be detected in vitro by various methods that are well known to those of ordinary skill in the art. For example, (1) cytotoxic T lymphocytes can be incubated with radioactively labeled target cells and the lysis of these target cells detected by the release of radioactivity, (2) helper T lymphocytes can be incubated with antigens and antigen presenting cells and the synthesis and secretion of cytokines measured by standard methods (Windhagen A; et al., 1995, Immunity 2(4): 373–80), (3) antigen presenting cells can be incubated with whole protein antigen and the presentation of that antigen on MHC detected by either T lymphocyte activation assays or biophysical methods (Harding et al., 1989, Proc. Natl. Acad. Sci., 86: 4230–4), (4) mast cells can be incubated with reagents that cross-link their Fc-epsilon receptors and histamine release measured by enzyme immunoassay (Siraganian, et al., 1983, TIPS 4: 432–437).

Similarly, products of an immune response in either a model organism (e.g., mouse) or a human patient can also be detected by various methods that are well known to those of ordinary skill in the art. For example, (1) the production of antibodies in response to vaccination can be readily detected by standard methods currently used in clinical laboratories, e.g., an ELISA; (2) the migration of immune cells to sites of inflammation can be detected by scratching the surface of skin and placing a sterile container to capture the migrating cells over scratch site (Peters et al., 1988, Blood 72: 1310–5); (3) the proliferation of peripheral blood mononuclear cells in response to mitogens or mixed lymphocyte reaction can be measured using ^{3}H-thymidine; (4) the phagocitic capacity of granulocytes, macrophages, and other phagocytes in PBMCs can be measured by placing PMBCs in wells together with labeled particles (Peters et al., 1988); and (5) the differentation of immune system cells can be measured by labeling PBMCs with antibodies to CD molecules such as CD4 and CD8 and measuring the fraction of the PBMCs expressing these markers.

As used herein, the phrase "signal transduction pathway" or "signal transduction event" refers to at least one biochemical reaction, but more commonly a series of biochemical reactions, which result from interaction of a cell with a stimulatory compound or agent. Thus, the interaction of a

stimulatory compound with a cell generates a "signal" that is transmitted through the signal transduction pathway, ultimately resulting in a cellular response, e.g., an immune response described above.

A signal transduction pathway refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. Signal transduction molecules of the present invention include, for example, MAb 147.1 of the invention. As used herein, the phrase "cell surface receptor" includes molecules and complexes of molecules capable of receiving a signal and the transmission of such a signal across the plasma membrane of a cell. An example of a "cell surface receptor" of the present invention is the T cell receptor (TCR) or the B7 ligands of CTLA-4.

A signal transduction pathway in a cell can be initiated by interaction of a cell with a stimulator that is inside or outside of the cell. If an exterior (i.e., outside of the cell) stimulator (e.g., an MHC-antigen complex on an antigen presenting cell) interacts with a cell surface receptor (e.g., a T cell receptor), a signal transduction pathway can transmit a signal across the cell's membrane, through the cytoplasm of the cell, and in some instances into the nucleus. If an interior (e.g., inside the cell) stimulator interacts with an intracellular signal transduction molecule, a signal transduction pathway can result in transmission of a signal through the cell's cytoplasm, and in some instances into the cell's nucleus.

Signal transduction can occur through, e.g., the phosphorylation of a molecule; non-covalent allosteric interactions; complexing of molecules; the conformational change of a molecule; calcium release; inositol phosphate production; proteolytic cleavage; cyclic nucleotide production and diacylglyceride production. Typically, signal transduction occurs through phosphorylating a signal transduction molecule.

The term "nonspecific T cell activation" refers to the stimulation of T cells independent of their antigenic specificity.

Production Of Human Antibodies To CTLA-4

The monoclonal antibodies (mAbs) and the human sequence antibodies of the invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology e.g., the standard somatic cell hybridization technique of Kohler and Milstein, *Nature* 256: 495 (1975). Any technique for producing monoclonal antibody can be employed e.g., viral or oncogenic transformation of B lymphocytes. One animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (e.g., murine myeloma cells) and fusion procedures are also known (see, e.g., Harlow and Lane (1988), *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor N.Y.).

Human monoclonal antibodies and human sequence antibodies directed against human CTLA-4 can be generated using transgenic mice carrying a human immune system rather than the mouse system. These transgenic mice, also referred to herein as "HuMAb-Mouse™", contain a human immunoglobulin gene miniloci that encodes unrearranged human heavy ($\mu$and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg, N. et al. (1994) *Nature* 368(6474): 856–859 and U.S. Pat. No. 5,770,429). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal (Lonberg, N. et al. (1994), supra; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49–101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* Vol. 13: 65–93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536–546). The preparation of transgenic mice is described in detail Section II below and in Taylor, L. et al. (1992) *Nucleic Acids Research* 20:6287–6295; Chen, J. et al. (1993) *International Immunology* 5: 647–656; Tuaillon et al. (1993) *Proc. Natl. Acad. Sci USA* 90:3720–3724; Choi et al. (1993). *Nature Genetics* 4:117–123; Chen, J. et al. (1993) EMBO J. 12: 821–830; Tuaillon et al. (1994) *J. Immunol.* 152:2912–2920; Lonberg et al., (1994) *Nature* 368(6474): 856–859; Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49–101; Taylor, L. et al. (1994) *International Immunology* 6: 579–591; Lonberg, N. and Huszar, D. (1995) Intern. Rev. Immunol. Vol. 13: 65–93; Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536–546; Fishwild, D. et al. (1996) *Nature Biotechnology* 14: 845–851. See further, U.S. Pat. Nos. 5,625,126 and 5,770,429, both to Lonberg and Kay, and GenPharm International; U.S. Pat. No. 5,545,807 to Surani et al.; International Publication Nos. WO 98/24884, published on Jun. 11, 1998; WO 94/25585, published Nov. 10, 1994; WO 93/1227, published Jun. 24, 1993; WO 92/22645, published Dec. 23, 1992; WO 92/03918, published Mar. 19, 1992. Alternatively, the CMD and HCo12 transgenes, described in Examples 1 and 2, below, can be used to generate human anti-CTLA-4 antibodies.

Detailed procedures to generate fully human monoclonal antibodies to CTLA-4 are described in the Examples below. Cumulative experience with various antigens has shown that the transgenic mice respond when initially immunized intraperitoneally (IP) with antigen in complete Freund's adjuvant, followed by every other week IP immunizations (up to a total of 6) with antigen in incomplete Freund's adjuvant. However, adjuvants other than Freund's are also found to be effective. In addition, whole cells in the absence of adjuvant are found to be highly immunogenic. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below), and mice with sufficient titers of anti-CTLA-4 human immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. It is expected that 2–3 fusions for each immunization may need to be performed. Between 6 and 24 mice are typically immunized for each antigen. Usually both HCo7 and HCo12 strains are used. In addition, both HCo7 and HCo12 transgene can be bred together into a single mouse having two different human heavy chain transgenes.

To purify human anti-CTLA-4 antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, N.J.). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD280 using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at −80° C.

To determine if the selected human anti-CTLA-4 monoclonal antibodies bind to unique epitopes, each antibody can

be biotinylated using commercially available reagents (Pierce, Rockford, Ill.). Competition studies using unlabeled monoclonal antibodies and biotinylated monoclonal antibodies can be performed using CTLA-4 coated-ELISA plates as described above. Biotinylated MAb binding can be detected with a strep-avidin-alkaline phosphatase probe.

To determine the isotype of purified antibodies, isotype ELISAs can be performed. Wells of microtiter plates can be coated with 1 μg/ml of anti-human IgG overnight at 4° C. After blocking with 1% BSA, the plates are reacted with 1 μg/ml or less of monoclonal antibodies or purified isotype controls, at ambient temperature for one to two hours. The wells can then be reacted with either human IgG1 or human IgM-specific alkaline phosphatase-conjugated probes. Plates are developed and analyzed as described above.

To demonstrate binding of monoclonal antibodies to live cells expressing the CTLA-4, flow cytometry can be used. Briefly, cell lines expressing CTLA-4 (grown under standard growth conditions) are mixed with various concentrations of monoclonal antibodies in PBS containing 0.1% BSA and 10% fetal calf serum, and incubated at 37° C. for 1 hour. After washing, the cells are reacted with Fluorescein-labeled anti-human IgG antibody under the same conditions as the primary antibody staining. The samples can be analyzed by FACScan instrument using light and side scatter properties to gate on single cells. An alternative assay using fluorescence microscopy may be used (in addition to or instead of) the flow cytometry assay. Cells can be stained exactly as described above and examined by fluorescence microscopy. This method allows visualization of individual cells, but may have diminished sensitivity depending on the density of the antigen.

Anti-CTLA-4 human IgGs can be further tested for reactivity with CTLA-4 antigen by Western blotting. Briefly, cell extracts from cells expressing CTLA-4 can be prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens are transferred to nitrocellulose membranes, blocked with 10% fetal calf serum, and probed with the monoclonal antibodies to be tested. Human IgG binding can be detected using anti-human IgG alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, Mo.).

Production of Transgenic Non-Human Animals that Generate Human Monoclonal Anti-CTLA-4 Antibodies

The present invention also provides transgenic non-human animals, e.g. a transgenic mice, which are capable of expressing human monoclonal antibodies that specifically bind to CTLA-4. High affinity human sequence antibodies are also provided. Some transgenic non-human animals, e.g., the transgenic mice, have a genome comprising a human heavy chain transgene and a light chain transgene. Some transgenic non-human animals are immunized with a purified or enriched preparation of CTLA-4 antigen and/or cells expressing CTLA-4. Some transgenic non-human animals are capable of producing multiple isotypes of human monoclonal antibodies to CTLA-4 (e.g., IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, e.g., classical or non-classical isotype switching.

The design of a transgenic non-human animal that responds to foreign antigen stimulation with a heterologous antibody repertoire, requires that the heterologous immunoglobulin transgenes contained within the transgenic animal function correctly throughout the pathway of B-cell development. In some mice, correct function of a heterologous heavy chain transgene includes isotype switching. Accordingly, the transgenes of the invention are constructed so as to produce isotype switching and one or more of the following: (1) high level and cell-type specific expression, (2) functional gene rearrangement, (3) activation of and response to allelic exclusion, (4) expression of a sufficient primary repertoire, (5) signal transduction, (6) somatic hypermutation, and (7) domination of the transgene antibody locus during the immune response.

Not all of the foregoing criteria need be met. For example, in transgenic animal in which the endogenous immunoglobulin loci of the transgenic animals are functionally disrupted, the transgene need not activate allelic exclusion. Further, in transgenic animals in which the transgene comprises a functionally rearranged heavy and/or light chain immunoglobulin gene, the second criteria of functional gene rearrangement is unnecessary, at least for that transgene which is already rearranged. For background on molecular immunology, See, e.g., *Fundamental Immunology,* 4th edition (1998), Paul, William E., ed. Lippencott-Raven Press, N.Y.

Some transgenic non-human animals used to generate the human monoclonal antibodies of the invention contain rearranged, unrearranged or a combination of rearranged and unrearranged heterologous immunoglobulin heavy and light chain transgenes in the germline of the transgenic animal. Each of the heavy chain transgenes comprises at least one CH gene. In addition, the heavy chain transgene can contain functional isotype switch sequences, which are capable of supporting isotype switching of a heterologous transgene encoding multiple CH genes in the B-cells of the transgenic animal. Such switch sequences can be those which occur naturally in the germline immunoglobulin locus from the species that serves as the source of the transgene CH genes, or such switch sequences can be derived from those which occur in the species that is to receive the transgene construct (the transgenic animal). For example, a human transgene construct that is used to produce a transgenic mouse may produce a higher frequency of isotype switching events if it incorporates switch sequences similar to those that occur naturally in the mouse heavy chain locus, as presumably the mouse switch sequences are optimized to function with the mouse switch recombinase enzyme system, whereas the human switch sequences are not. Switch sequences can be isolated and cloned by conventional cloning methods, or can be synthesized de novo from overlapping synthetic oligonucleotides designed on the basis of published sequence information relating to immunoglobulin switch region sequences (Mills et al., *Nucl. Acids Res.* 15:7305–7316 (1991); Sideras et al., *Intl. Immunol.* 1:631–642 (1989).

For each of the foregoing transgenic animals, functionally rearranged heterologous heavy and light chain immunoglobulin transgenes are found in a significant fraction of the B-cells of the transgenic animal (at least 10 percent).

The transgenes used to generate the transgenic animals of the invention include a heavy chain transgene comprising DNA encoding at least one variable gene segment, one diversity gene segment, one joining gene segment and at least one constant region gene segment. The immunoglobulin light chain transgene comprises DNA encoding at least one variable gene segment, one joining gene segment and at least one constant region gene segment. The gene segments encoding the light and heavy chain gene segments are heterologous to the transgenic non-human animal in that they are derived from, or correspond to, DNA encoding immunoglobulin heavy and light chain gene segments from a species not consisting of the transgenic non-human animal.

In one aspect of the invention, the transgene is constructed such that the individual gene segments are unrearranged, i.e., not rearranged so as to encode a functional immunoglobulin light or heavy chain. Such unrearranged transgenes support recombination of the V, D, and J gene segments (functional rearrangement) and preferably support incorporation of all or a portion of a D region gene segment in the resultant rearranged immunoglobulin heavy chain within the transgenic non-human animal when exposed to CTLA-4 antigen.

Such transgenes typically comprise a substantial portion of the C, D, and J segments as well as a subset of the V gene segments. In such transgene constructs, the various regulatory sequences, e.g. promoters, enhancers, class switch regions, splice-donor and splice-acceptor sequences for RNA processing, recombination signals and the like, comprise corresponding sequences derived from the heterologous DNA. Such regulatory sequences may be incorporated into the transgene from the same or a related species of the non-human animal used in the invention. For example, human immunoglobulin gene segments may be combined in a transgene with a rodent immunoglobulin enhancer sequence for use in a transgenic mouse. Alternatively, synthetic regulatory sequences may be incorporated into the transgene, wherein such synthetic regulatory sequences are not homologous to a functional DNA sequence that is known to occur naturally in the genomes of mammals. Synthetic regulatory sequences are designed according to consensus rules, such as, for example, those specifying the permissible sequences of a splice-acceptor site or a promoter/enhancer motif. The transgene may comprise a minilocus.

Some transgenic animals used to generate human antibodies to CTLA-4 contain at least one, typically 2–10, and sometimes 25–50 or more copies of the transgene described in Example 37 of U.S. Pat. No. 5,770,429, or the transgene described in Example 2 below (e.g., HCo12), at least one copy of a light chain transgene described in Examples 38 of U.S. Pat. No. 5,770,429, two copies of the Cmu deletion described in Example 1 below, and two copies of the Jkappa deletion described in Example 9 of U.S. Pat. No. 5,770,429. The resultant animals are injected with antigens and used for production of human monoclonal antibodies against these antigens.

Some transgenic animals exhibit immunoglobulin production with a significant repertoire, ideally substantially similar to that of a native mouse. Thus, for example, animals in which the endogenous Ig genes have been inactivated, the total immunoglobulin levels range from about 0.1 to about 10 mg/ml of serum.

The immunoglobulins expressed by the transgenic mice typically recognize about one-half or more of highly antigenic proteins, e.g., staphylococcusprotein A. Typically, the immunoglobulins exhibit an association constant for preselected antigens of at least about 10^7M^{-1}, 10^9M^{-1}, 10^9M^{-1}, $10^{10}M^{-1}$, 10^{11} M^{-1}, $10^{12}M^{-1}$, $10^{13}M^{-1}$, or greater.

The transgenic mice of the present invention can be immunized with a purified or enriched preparation of human CTLA-4 antigen (or antigenic fragment thereof) and/or cells expressing human CTLA-4 as described previously. The mice produce B cells that undergo class-switching via intratransgene switch recombination (cis-switching) and express immunoglobulins reactive with CTLA-4. The immunoglobulins can be human sequence antibodies, wherein the heavy and light chain polypeptides are encoded by human transgene sequences, which may include sequences derived by somatic mutation and V region recombinatorial joints, as well as germline-encoded sequences; these human sequence immunoglobulins can be referred to as being substantially identical to a polypeptide sequence encoded by a human VL or VH gene segment and a human JL or JH segment, even though other non-germline sequences may be present as a result of somatic mutation and differential V-J and V-D-J recombination joints. With respect to such human sequence antibodies, the variable regions of each chain are typically at least 80 percent encoded by human germ line V, J, and, in the case of heavy chains, D, gene segments; frequently at least 85 percent of the variable regions are encoded by human germline sequences present on the transgene; often 90 or 95 percent or more of the variable region sequences are encoded by human germline sequences present on the transgene. However, since non-germline sequences are introduced by somatic mutation and VJ and VDJ joining, the human sequence antibodies frequently have some variable region sequences (and less frequently constant region sequences) which are not encoded by human V, D, or J gene segments as found in the human transgene(s) in the germline of the mice. Typically, such non-germline sequences (or individual nucleotide positions) cluster in or near CDRs, or in regions where somatic mutations are known to cluster. The human sequence antibodies which bind to the predetermined antigen can result from isotype switching, such that human antibodies comprising a human sequence γ chain (such as γ1, γ2, γ3, or γ4) and a human sequence light chain (such as kappa or lambda) are produced. Such isotype-switched human sequence antibodies often contain one or more somatic mutation(s), typically in the variable region and often in or within about 10 residues of a CDR) as a result of affinity maturation and selection of B cells by antigen, particularly subsequent to secondary (or subsequent) antigen challenge. Some high affinity human sequence antibodies have equilibrium association constants of at least about 1×10^7 M^{-1}, or at least about 1×10^8 M^{-1}, or more than about 1×10^9 M^{-1}, or 5×10^9 M^{-1} to 1×10^{11} M^{-1} or greater.

Another aspect of the invention pertains to the B cells from such mice which can be used to generate hybridomas expressing human monoclonal antibodies which bind with high affinity (e.g., having association constant of greater than 10^7M^{-1}) to CTLA-4. These hybridomas are used to generate a composition comprising an immunoglobulin having an association constant (Ka) of at least 10^7 M^{-1} for binding CTLA-4. Such immunoglobulin contains a human sequence light chain composed of a light chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human Vk or Vλ gene segment and a human Jk or Jλ segment, and a light chain constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human Ck or Cλ gene segment. It also contains a human sequence heavy chain composed of a heavy chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human VH gene segment, optionally a D region, and a human JH segment, and a constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human CH gene segment.

The invention also provides human monoclonal antibodies and human sequence antibodies to human CTLA-4 derivatized or linked to another functional molecule, e.g., another peptide or protein (e.g., a cytokine, a cytotoxic agent, an immune stimulatory or inhibitory agent, a Fab' fragment, and the like, as discussed above) to generate a bispecific or multispecific molecule which binds to multiple binding sites or target epitopes. For example, an antibody or

antigen-binding portion of the invention can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic.

Accordingly, the present invention includes bispecific and multispecific composition comprising at least one human sequence antibody or antigen binding fragment with a first binding specificity for human CTLA-4 and a second binding specificity for a second target epitope. The second target epitope can be an Fc receptor, e.g., human FcγRI or a human Fcγ receptor. Therefore, the invention includes bispecific and multispecific molecules capable of binding both to FcγRI, FcγR or FcεR expressing effector cells (e.g., monocytes, macrophages or polymorphonuclear cells (PMNs)), and to target cells expressing human CTLA-4. These multi-specific (e.g., bispecific or multispecific) molecules target human CTLA-4 expressing cells to effector cells, and trigger Fc receptor-mediated effector cell activities, such as phagocytosis of a human CTLA-4-expressing cells, antibody dependent cell-mediated cytotoxicity (ADCC), cytokine release, or generation of superoxide anion.

The bispecific and multispecific molecules of the invention can comprise a binding specificity at least one antibody, or an antibody fragment thereof, including, e.g., an Fab, Fab', F(ab')2, Fv, or a single chain Fv. The antibody may also be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in, e.g, Ladner et al. U.S. Pat. No. 4,946,778. Bispecific and multispecific molecules of the invention can comprise a binding specificity for an FcyR or an FcγR present on the surface of an effector cell, and a second binding specificity for a target cell antigen, e.g., human CTLA-4.

The binding specificity for an Fc receptor is provided by a monoclonal antibody, the binding of which is not blocked by human immunoglobulin G (IgG). As used herein, the term "IgG receptor" refers to any of the eight γ-chain genes located on chromosome 1. These genes encode a total of twelve transmembrane or soluble receptor isoforms which are grouped into three Fcγ receptor classes: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). For example, the Fcγ receptor can be a human high affinity FcγRI. The human FcγRI is a 72 kDa molecule, which shows high affinity for monomeric IgG (10^8 to $10^9 M^{-1}$).

The production and characterization of these preferred monoclonal antibodies are described by Fanger et al. in PCT application WO 88/00052 and in U.S. Pat. No. 4,954,617. These antibodies bind to an epitope of FcγRI, FcγRII or FcγRIII at a site which is distinct from the Fcγ binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this invention are MAb 22, MAb 32, MAb 44, MAb 62 and MAb 197. The hybridoma producing MAb 32 is available from the American Type Culture Collection, ATCC Accession No. HB9469. Anti-FcγRI MAb 22, $F(ab')_2$ fragments of MAb 22, and can be obtained from Medarex, Inc. (Annandale, N.J.). In other embodiments, the anti-Fc γ receptor antibody is a humanized form of monoclonal antibody 22 (H22). The production and characterization of the H22 antibody is described in Graziano (1995) J. Immunol 155:4996–5002 and PCT/US93/10384. The H22 antibody producing cell line was deposited at the American Type Culture Collection on Nov. 4, 1992 under the designation HA022CL1 and has the accession no. CRL 11177.

The binding specificity for an Fc receptor can also be provided by an antibody that binds to a human IgA receptor, e.g., an Fc-alpha receptor (FcαRI (CD89)). Preferably, the antibody binds to a human IgA receptor at a site that is not blocked by endogenous IgA. The term "IgA receptor" is intended to include the gene product of one α-gene (FcαRI) located on chromosome 19. This gene is known to encode several alternatively spliced transmembrane isoforms of 55 to 110 kDa. FcαRI (CD89) is constitutively expressed on monocytes/macrophages, eosinophilic and neutrophilic granulocytes, but not on non-effector cell populations. FcαRI has medium affinity ($\approx 5\times10^7 M^{-1}$) for both IgA1 and IgA2, which is increased upon exposure to cytokines such as G-CSF or GM-CSF (Morton (1996) Critical Reviews in Immunology 16:423–440). Four FcαRI-specific monoclonal antibodies, identified as A3, A59, A62 and A77, which bind FcαRI outside the IgA ligand binding domain, have been described by, e.g, Monteiro (1992) *J. Immunol.* 148:1764.

Bispecific and multispecific molecules of the invention can further comprise a binding specificity which recognizes, e.g., binds to, a target cell antigen, e.g. human CTLA-4. The binding specificity is provided by a human sequence antibody or a human monoclonal antibody of the present invention.

An "effector cell specific antibody" as used herein refers to an antibody or functional antibody fragment that binds the Fc receptor of effector cells. Preferred antibodies for use in the subject invention bind the Fc receptor of effector cells at a site which is not bound by endogenous immunoglobulin.

As used herein, the term "effector cell" refers to an immune cell which is involved in the effector phase of an immune response, as opposed to the cognitive and activation phases of an immune response. Exemplary immune cells include a cell of a myeloid or lymphoid origin, e.g., lymphocytes (e.g., B cells and T cells including cytolytic T cells (CTLs)), killer cells, natural killer cells, macrophages, monocytes, eosinophils, neutrophils, polymorphonuclear cells, granulocytes, mast cells, and basophils. Effector cells express specific Fc receptors and carry out specific immune functions. An effector cell can induce antibody-dependent cell-mediated cytotoxicity (ADCC), e.g., a neutrophil capable of inducing ADCC. For example, monocytes, macrophages, neutrophils, eosinophils, and lymphocytes which express FcαR are involved in specific killing of target cells and presenting antigens to other components of the immune system, or binding to cells that present antigens. An effector cell can also phagocytose a target antigen, target cell, or microorganism.

The expression of a particular FcR on an effector cell can be regulated by humoral factors such as cytokines. For example, expression of FcγRI has been found to be up-regulated by interferon gamma (IFN-γ). This enhanced expression increases cytotoxic activity (including, e.g., phagocytosis) by FcγRI-bearing cells against target cells.

"Target cell" shall mean any undesirable cell in a subject (e.g., a human or animal) that can be targeted by a composition (e.g., a human sequence antibody or a human monoclonal antibody of the invention, a bispecific or a multispecific molecule of the invention). The target cell can be a cell expressing or overexpressing human CTLA-4. Cells expressing human CTLA-4 can include tumor cells, e.g. lymphomas.

In addition to human sequence antibodies and human monoclonal antibodies of the invention, other antibodies can be also be employed in the bispecific or multispecific molecules of the invention, including, e.g., murine, chimeric and humanized monoclonal antibodies.

Chimeric mouse-human monoclonal antibodies (i.e., chimeric antibodies) can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted. (See, e.g., Robinson et al., International Patent Publication PCT/US86/02269; Akira, et al., European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Application WO 86/01533; Cabilly et al. U.S. Pat. No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better (1988) Science 240:1041–1043; Liu (1987) PNAS 84:3439–3443; Liu (1987) J. Immunol. 139:3521–3526; Sun (1987) PNAS 84:214–218; Nishimura (1987) Canc. Res. 47:999–1005; Wood (1985) Nature 314:446–449; Shaw (1988) J. Natl. Cancer Inst. 80:1553–1559).

The chimeric antibody can be further humanized by replacing sequences of the Fv variable region which are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General reviews of humanized chimeric antibodies are provided by Morrison (1985) *Science* 229:1202–1207 and by Oi (1986) *BioTechniques* 4:214. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from 7E3, an anti-$GPII_bIII_a$ antibody producing hybridoma. The recombinant DNA encoding the chimeric antibody, or fragment thereof, can then be cloned into an appropriate expression vector. Suitable humanized antibodies can alternatively be produced by CDR substitution U.S. Pat. No. 5,225,539; Jones (1986) Nature 321:552–525; Verhoeyan et al. 1988 Science 239:1534; and Beidler (1988) J. Immunol. 141:4053–4060.

All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to the Fc receptor. An antibody can be humanized by any method, which is capable of replacing at least a portion of a CDR of a human antibody with a CDR derived from a non-human antibody. Winter describes a method which may be used to prepare the humanized antibodies of the present invention, see UK Patent Application GB 2188638A, filed on Mar. 26, 1987. The human CDRs may be replaced with non-human CDRs using oligonucleotide site-directed mutagenesis as described in, e.g., WO 94/10332 entitled, Humanized Antibodies to Fc Receptors for Immunoglobulin G on Human Mononuclear Phagocytes.

Chimeric and humanized antibodies in which specific amino acids have been substituted, deleted or added are also within the scope of the invention. For example, humanized antibodies can have amino acid substitutions in the framework region, such as to improve binding to the antigen. In a humanized antibody having mouse CDRs, amino acids located in the human framework region can be replaced with the amino acids located at the corresponding positions in the mouse antibody. Such substitutions are known to improve binding of humanized antibodies to the antigen in some instances. Antibodies in which amino acids have been added, deleted, or substituted are referred to herein as modified antibodies or altered antibodies.

Bispecific and multispecific molecules of the invention can further include a third binding specificity, in addition to an anti-Fc binding specificity and an anti-human CTLA-4 binding specificity. The third binding specificity can be an anti-enhancement factor (EF) portion, e.g., a molecule which binds to a surface protein involved in cytotoxic activity and thereby increases the immune response against the target cell. The "anti-enhancement factor portion" can be an antibody, functional antibody fragment or a ligand that binds to a given molecule, e.g., an antigen or a receptor, and thereby results in an enhancement of the effect of the binding determinants for the Fc receptor or target cell antigen. The "anti-enhancement factor portion" can bind an Fc receptor or a target cell antigen. Alternatively, the anti-enhancement factor portion can bind to an entity that is different from the entity to which the first and second binding specificities bind. For example, the anti-enhancement factor portion can bind a cytotoxic T-cell via, e.g., CD2, CD3, CD8, CD28, CD4, CD40, ICAM-1 or other immune cell molecules that are involved in an increased immune response against the target cell.

Bispecific and multispecific molecules of the present invention can be made using chemical techniques (see, e.g., Kranz (1981) *Proc. Natl. Acad. Sci. USA* 78:5807), "polydoma" techniques (see, e.g., U.S. Pat. No. 4,474,893), or recombinant DNA techniques. Bispecific and multispecific molecules of the present invention can also be prepared by conjugating the constituent binding specificities, e.g., the anti-FcR and anti-human CTLA-4 binding specificities, using methods known in the art and as described herein. For example, each binding specificity of the bispecific and multispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohaxane-1-carboxylate (sulfo-SMCC) (see, e.g., Karpovsky (1984) J. Exp. Med. 160:1686; Liu (1985) Proc. Natl. Acad. Sci. USA 82:8648). Other methods include those described by Paulus (Behring Ins. Mitt. (1985) No. 78, 118–132; Brennan (1985) Science 229:81–83), Glennie (1987) J. Immunol. 139: 2367–2375). Other conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, Ill.).

When the binding specificities are antibodies (e.g., two humanized antibodies), they can be conjugated via sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. The hinge region can be modified to contain an odd number of sulfhydryl residues, e.g., one, prior to conjugation.

Alternatively, both binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the bispecific and multispecific molecule is a MAb×MAb, MAb×Fab, Fab×$F(ab')_2$ or ligand×Fab fusion protein. A bispecific and multispecific molecule of the invention, e.g., a bispecific molecule can be a single chain molecule, such as a single chain bispecific antibody, a single chain bispecific molecule comprising one single chain antibody and a binding determinant, or a single chain bispecific molecule comprising two binding determinants. Bispecific and multispecific molecules can also be single chain molecules or may comprise at least two single chain molecules. Methods for preparing bi- and multispecific molecules are described for

example in U.S. Pat. No. 5,260,203; U.S. Pat. No. 5,455,030; U.S. Pat. No. 4,881,175; U.S. Pat. No. 5,132,405; U.S. Pat. No. 5,091,513; U.S. Pat. No. 5,476,786; U.S. Pat. No. 5,013,653; U.S. Pat. No. 5,258,498; and U.S. Pat. No. 5,482,858.

Binding of the bispecific and multispecific molecules to their specific targets can be confirmed by enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay (RIA), or a Western Blot Assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (e.g., an antibody) specific for the complex of interest. For example, the FcR-antibody complexes can be detected using e.g., an enzyme-linked antibody or antibody fragment which recognizes and specifically binds to the antibody-FcR complexes. Alternatively, the complexes can be detected using any of a variety of other immunoassays. For example, the antibody can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a y counter or a scintillation counter or by autoradiography.

Also included in the invention are modified antibodies. The term "modified antibody" includes antibodies, such as monoclonal antibodies, chimeric antibodies, and humanized antibodies which have been modified by, e.g., deleting, adding, or substituting portions of the antibody. For example, an antibody can be modified by deleting the constant region and replacing it with a constant region meant to increase half-life, e.g., serum half-life, stability or affinity of the antibody.

The antibody conjugates of the invention can be used to modify a given biological response or create a biological response (e.g., to recruit effector cells). The drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-alpha; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243–56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623–53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475–506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303–16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119–58 (1982).

Pharmaceutical Compositions

The invention provides pharmaceutical compositions comprising one or a combination of human monoclonal antibodies and/or human sequence antibodies (intact or binding fragments) formulated together with a pharmaceutically acceptable carrier. Some compositions include a combination of multiple (e.g., two or more) isolated human antibodies and/or human sequence antibody or antigen-binding portions thereof of the invention. In some compositions, each of the antibodies or antigen-binding portions thereof of the composition is a monoclonal antibody or a human sequence antibody that binds to a distinct, pre-selected epitope of human CTLA-4.

A. Effective Dosages

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors.

A physician or veterinarian can start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required to achieve the

desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a compositions of the invention is that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose generally depends upon the factors described above. It is preferred that administration be intravenous, intramuscular, intraperitoneal, or subcutaneous, or administered proximal to the site of the target. If desired, the effective daily dose of a therapeutic compositions can be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

Effective doses of the compositions of the present invention, for the treatment of immune-related conditions and diseases described herein vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Treatment dosages need to be titrated to optimize safety and efficacy.

For administration with an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1–10 mg/kg. An exemplary treatment regime entails administration once per every two weeks or once a month or once every 3 to 6 months. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to CTLA-4 in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of 1–1000 μg/ml and in some methods 25–300 μg/ml. Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In generals human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patent can be administered a prophylactic regime.

Doses for nucleic acids encoding immunogens range from about 10 ng to 1 g, 100 ng to 100 mg, 1 μg to 10 mg, or 30–300 μg DNA per patient. Doses for infectious viral vectors vary from 10–100, or more, virions per dose.

Some human sequence antibodies and human monoclonal antibodies of the invention can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, See, e.g., U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (See, e.g., V. V. Ranade (1989) *J. Clin. Pharmacol.* 29:685. Exemplary targeting moieties include folate or biotin (See, e.g., U.S. Pat. No. 5,416,016 to Low et al.); mannosides (Umezawa et al., (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P. G. Bloeman et al. (1995) *FEBS Lett.* 357:140; M. Owais et al. (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe et al. (1995) *Am. J. Physiol.* 1233:134), different species of which may comprise the formulations of the inventions, as well as components of the invented molecules; p120 (Schreier et al. (1994) *J. Biol. Chem.* 269:9090); See also K. Keinanen; M. L. Laukkanen (1994) *FEBS Lett.* 346:123; J. J. Killion; I. J. Fidler (1994) *Immunomethods* 4:273. In some methods, the therapeutic compounds of the invention are formulated in liposomes; in a more preferred embodiment, the liposomes include a targeting moiety. In some methods, the therapeutic compounds in the liposomes are delivered by bolus injection to a site proximal to the tumor or infection. The composition should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms such as bacteria and fungi.

For therapeutic applications, the pharmaceutical compositions are administered to a patient suffering from established disease in an amount sufficient to arrest or inhibit further development or reverse or eliminate, the disease, its symptoms or biochemical markers. For prophylactic applications, the pharmaceutical compositions are administered to a patient susceptible or at risk of a disease in an amount sufficient to delay, inhibit or prevent development of the disease, its symptoms and biochemical markers. An amount adequate to accomplish this is defined as a "therapeutically-" or "prophylactically-effective dose." Dosage depends on the disease being treated, the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected. Specifically, in treatment of tumors, a "therapeutically effective dosage" can inhibit tumor growth by at least about 20%, or at least about 40%, or at least about 60%, or at least about 80% relative to untreated subjects. The ability of a compound to inhibit cancer can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit by conventional assays in vitro. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject.

The composition should be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier can be an isotonic buffered saline solution, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition. Long-term absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

When the active compound is suitably protected, as described above, the compound may be orally administered, for example, with an inert diluent or an assimilable edible carrier.

B. Routes of Administration

Pharmaceutical compositions of the invention also can be administered in combination therapy, i.e., combined with other agents. For example, in treatment of cancer, the combination therapy can include a composition of the present invention with at least one anti-tumor agent or other conventional therapy, such as radiation treatment.

Pharmaceutically acceptable carriers includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier can be suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound, i.e., antibody, bispecific and multispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (See, e.g., Berge, S. M., et al. (1977) J. Pharm. Sci. 66:1–19).

Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A composition of the present invention can be administered by a variety of methods known in the art. The route and/or mode of administration vary depending upon the desired results. The active compounds can be prepared with carriers that protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are described by e.g., *Sustained and Controlled Release Drug Delivery Systems*, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. Pharmaceutical compositions are preferably manufactured under GMP conditions.

To administer a compound of the invention by certain routes of administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan et al. (1984) *J. Neuroimmunol.* 7:27).

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile, substantially isotonic, and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Therapeutic compositions can also be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in, e.g., U.S. Pat. Nos. 5,399,163, 5,383,851, 5,312,335, 5,064,413, 4,941,880, 4,790,824, or 4,596,556. Examples of implants and modules useful in the present invention include: U.S. Pat. No. 4,487, 603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Pat. No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Pat. No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Pat. No. 4,447, 224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Pat. No. 4,439, 196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Pat. No. 4,475, 196, which discloses an osmotic drug delivery system. Many other such implants, delivery systems, and modules are known.

C. Formulation

For the therapeutic compositions, formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations can conveniently be presented in unit dosage form and may be prepared by any methods known in the art of pharmacy. The

amount of active ingredient which can be combined with a carrier material to produce a single dosage form vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred percent, this amount range from about 0.01 percent to about ninety-nine percent of active ingredient, from about 0.1 percent to about 70 percent, or from about 1 percent to about 30 percent.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate. Dosage forms for the topical or transdermal administration of compositions of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The phrases "parenteral administration" and "administered parenterally" mean modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given alone or as a pharmaceutical composition containing, for example, 0.01 to 99.5% (or 0.1 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

Methods and uses of the Invention

A. Methods

The compositions (e.g., human sequence antibodies and human monoclonal antibodies to human CTLA-4 and derivatives/conjugates thereof) of the present invention have in vitro and in vivo diagnostic and therapeutic utilities. For example, these molecules can be administered to cells in culture, e.g. in vitro or ex vivo, or in a subject, e.g., in vivo, to treat, prevent or diagnose a variety of disorders. The term "subject" includes human and non-human animals. Non-human animals includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, and reptiles. The methods are particularly suitable for treating human patients having a disorder that can be treated by augmenting or reducing the T-cell mediated immune response.

When antibodies to CTLA-4 are administered together with another agent, the two can be administered in either order or simultaneously. The methods can be used to treat any kind of cancer including melanoma, colon cancer, prostate cancer, and renal cancer.

For example, latex microspheres coated with anti-CTLA-4 (to increase the valency of the antibody) can inhibit T cell proliferation and activation. Agents having the same antibody combining site may act as a CTLA-4 antagonist when presented as an Fab or a soluble IgG, and a CTLA-4 agonist when highly cross-linked. Thus multivalent forms of anti-CTLA-4 antibodies are useful therapeutic agents for down-modulating immune responses.

In addition to linking to latex microspheres or other insoluble particles, the antibodies can be cross-linked to each other or genetically engineered to form multimers. Cross-linking can be by direct chemical linkage, or by indirect linkage such as an antibody-biotin-avidin complex. Cross-linking can be covalent, where chemical linking groups are employed, or non-covalent, where protein-protein or other protein-ligand interactions are employed. Genetic engineering approaches for linking include, e.g., the re-expression of the variable regions of high-affinity IgG antibodies in IgM expression vectors or any protein moiety (e.g., polylysine, and the like). Converting a high affinity IgG antibody to an IgM antibody can create a decavalent complex with very high avidity. IgA_2 expression vectors may also be used to produce multivalent antibody complexes. IgA_2 can form polymers together with J chain and secretory component. IgA2 may have the added advantage that it can be additionally crosslinked by the IgA receptor CD89, which is expressed on neutrophils, macrophages, and monocytes.

Agonism can also be obtained using some preparations of polyclonal antibodies to CTLA-4 comprising antibodies to at least two non-overlapping epitopes on CTLA-4. One antibody in such a preparation containing two binding sites can bind to two molecules of CTLA-4 to form a small cluster. A second antibody possessing different binding sites can then link (aggregate) these small clusters to form large clusters, thereby forming a complex of CTLA-4 (on the cell surface) that can transduce a signal to the T cell to inhibit, reduce or prevent activation of the T-cell bearing (expressing) CTLA-4. Thus, some preparations of polyclonal antibodies show similar agonism to the polyvalent preparations described above.

Therefore, polyvalent or polyclonal preparations of anti CTLA-4 antibodies are useful for agonizing the CTLA-4 receptor, thereby suppressing immune responses otherwise mediated by T cells bearing the CTLA-4 receptor. Some examples of diseases that can be treated using such polyvalent or polyclonal preparations of antibodies induce autoimmune disease, transplant rejection, and inflammation.

B. Uses

1. Activating Immune Responses

a. Cancer

Some therapeutic methods treat patients with cancer. Blockade of CTLA-4 by antibodies can enhance the immune response to cancerous cells in the patient. Optionally, antibodies to CTLA-4 can be combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines and cell surface antigens such as B7 (see, e.g., Hurwitz, A. et al (1998) *Proc. Natl. Acad. Sci U.S.A.* 95, 10067–10071).

In murine experimental systems, implantation of some tumors followed by the administration of anti-CTLA-4 antibodies can result in the rejection of tumors. In some cases tumor rejection of established tumors occurs; in other cases the growth of the tumor is slowed by the use of anti-CTLA-4 antibodies. In general CTLA-4 blockade is effective against immunogenic tumors. Operationally this is defined as those tumors for which vaccination using the tumor itself can lead to immunity to tumor challenge. In humans, some tumors have been shown to be immunogenic such as melanomas. It is anticipated that by raising the threshold of T cell activation by CTLA-4 blockade, we may expect to activate tumor responses in the host.

CTLA-4 blockade is most effective when combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised (see Rosenberg, S., 2000, Development of Cancer Vaccines, ASCO *Educational Book* Spring: 60–62; Logothetis, C., 2000, ASCO *Educational Book* Spring: 300–302; Khayat, D. 2000, ASCO *Educational Book* Spring: 414–428; Foon, K. 2000, ASCO *Educational Book* Spring: 730–738; see also Restifo, N. and Sznol, M., Cancer Vaccines, Ch. 61, pp. 3023–3043 in DeVita, V. et al. (eds.), 1997, *Cancer: Principles and Practice of Oncology*, Fifth Edition). In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. These cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff et al. (1993) *Proc. Natl. Acad. Sci U.S.A.* 90 (80: 3539–43).

Anti-CTLA-4 blockade together with the use of GMCSF-modified tumor cell vaccines has been shown to be effective in a number of experimental tumor models such as mammary carcinoma (Hurwitz et al. (1998) supra), primary prostate cancer (Hurwitz A. et al. (2000) *Cancer Research* 60 (9): 2444–8) and melanoma (van Elsas, A et al. (1999) *J. Exp. Med.* 190: 355–66). In these instances, non-immunogenic tumors, such as the B16 melanoma, have been rendered susceptible to destruction by the immune system. The tumor cell vaccine may also be modified to express other immune activators such as IL2, and costimulatory molecules, among others.

The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, SA (1999) *Immunity* 10: 281–7). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp 100, MAGE antigens, Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. CTLA-4 blockade may be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self antigens and are therefore tolerant to them. The tumor antigen may also include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim, N et al. (1994) *Science* 266, 2011–2013). (These somatic tissues may be protected from immune attack by various means). Tumor antigen may also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (ie. ber-abl in the Philadelphia chromosome), or idiotype from B cell tumors. Other tumor vaccines may include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which may be used in conjunction with CTLA-4 blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot, R & Srivastava, P (1995) *Science* 269: 1585–1588; Tamura, Y. et al. (1997) *Science* 278: 117–120.

Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced ex vivo and loaded with various protein and peptide antigens as well as tumor cell extracts (Nestle, F. et al. (1998) *Nature Medicine* 4: 328–332). DCs may also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler, A. et al. (2000) *Nature Medicine* 6:332–336). As a method of vaccination, DC immunization may be effectively combined with CTLA-4 blockade to activate more potent anti-tumor responses.

CTLA-4 blockade may also be combined with standard cancer treatments. CTLA-4 blockade may be effectively combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr, M. et al. (1998) *Cancer Research* 58: 5301–5304). The scientific rationale behind the combined use of CTLA-4 blockade and chemotherapy is that cell death, that is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with CTLA-4 blockade through cell death are radiation, surgery, and hormone deprivation (Kwon, E. et al. (1999) *Proc. Nail. Acad. Sci U.S.A.* 96 (26): 15074–9. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with CTLA-4 blockade. Inhibition of angiogenesis leads to tumor cell death which may feed tumor antigen into host antigen presentation pathways.

CTLA-4 blocking antibodies can also be used in combination with bispecific antibodies that target Fc alpha or Fc gamma receptor-expressing effectors cells to tumor cells (see, e.g., U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used to target two separate antigens. For example anti-Fc receptor/anti tumor antigen (i.e., Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would by augmented by the use of CTLA-4

blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins which are expressed by the tumors and which are immunosuppressive. These include among others Tgfβ (Kehrl, J. et al. (1986) *J. Exp. Med.* 163: 1037–1050), IL-10 (Howard, M. & O'Garra, A. (1992) *Immunology Today* 13: 198–200), and Fas ligand (Hahne, M. et al. (1996) *Science* 274: 1363–1365). Antibodies to each of these entities may be used in combination with anti-CTLA-4 to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

Other antibodies which may be used to activate host immune responsiveness can be used in combination with anti-CTLA-4. These include molecules on the surface of dendritic cells which activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge, J. et al. (1998) *Nature* 393: 474–478). and can be used in conjuction with CTLA-4 antibodies (Ito, N. et al. (2000) *Immunobiology* 201 (5) 52740). Activating antibodies to T cell costimulatory molecules such as OX-40 (Weinberg, A. et al. (2000) *J Immunol* 164: 2160–2169), 4–1BB (Melero, I. et al. (1997) *Nature Medicine* 3: 682–685 (1997), and ICOS (Hutloff, A. et al. (1999) *Nature* 397: 262–266) may also provide for increased levels of T cell activation.

Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses. CTLA-4 blockade can be used to increase the effectiveness of the donor engrafted tumor specific T cells (Blazar, B. et al., (1999) *J Immunol* 162: 6368–6377).

There are also several experimental treatment protocols that involve ex vivo activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg, R. & Riddell, S. (1999) 285: 546–51). These methods may also be used to activate T cell responses to infectious agents such as CMV (see below). Ex vivo activation in the presence of anti-CTLA-4 antibodies may be expected to increase the frequency and activity of the adoptively transferred T cells.

b. Infectious Diseases

Other methods of the invention are used to treat patients that have been exposed to particular toxins or pathogens. Similar to its application to tumors as discussed above, antibody mediated CTLA-4 blockade can be used alone, or as an adjuvant, in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. CTLA-4 blockade has been shown to be effective in the acute phase of infections of *Nippostrongylus brasiliensis* (McCoy, K. et al. (1997) 186(2); 183–187) and *Leishmania donovani* (Murphy, M. et al. (1998) J. Immunol. 161:4153–4160). Examples of pathogens for which this therapeutic approach may be particularly useful, include pathogens for which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to HIV, Hepatitis (A, B, & C), Influenza, Herpes, *Giardia*, Malaria, *Leishmania, Staphylococcus Aureus, Pseudomonas aeruginosa*. CTLA-4 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of the infections. These novel epitopes are recognized as foreign at the time of anti-human CTLA-4 administration, thus provoking a strong T cell response that is not dampened by negative signals through CTLA-4.

Some examples of pathogenic viruses causing infections treatable by methods of the invention include hepatitis (A, B, or C), herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

Some examples of pathogenic bacteria causing infections treatable by methods of the invention include *chlamydia*, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, *klebsiella, proteus, serratia, pseudomonas, legionella*, diphtheria, *salmonella*, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lymes disease bacteria.

Some examples of pathogenic fungi causing infections treatable by methods of the invention include *Candida* (*albicans, krusei, glabrata, tropicalis*, etc.), *Cryptococcus neoformans, Aspergillus* (*fumigatus, niger*, etc.), Genus Mucorales (*Mucor, Absidia, Rhizophus*), *Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis* and *Histoplasma capsulatum.*

Some examples of pathogenic parasites causing infections treatable by methods of the invention include *Entamoeba histolytica, Balantidium coli, Naegleria fowleri, Acanthamoeba* sp., *Giardia lambia, Cryptosporidium* sp., *Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, Nippostrongylus brasiliensis.*

In all of the above methods, a CTLA-4 blockade can be combined with other forms of immunotherapy such as cytokine treatment (e.g. interferons, GM-CSF, GCSF, IL-2), or bispecific antibody therapy, which provides for enhanced presentation of tumor antigens (see, e.g., Holliger (1993) *Proc. Natl. Acad. Sci. USA* 90:6444–6448; Poljak (1994) *Structure* 2:1121–1123).

C. Promoting Beneficial "Autoimmune" Reactions for the Treatment of Disease and Therapeutic Intervention

The ability of anti-CTLA-4 antibodies to provoke and amplify autoimmune responses has been documented in a number of experimental systems (EAE—Experimental Autoimmune Encephalomyelitis, a murine model for MS (Perrin, P. et al. (1996) *J Immunol* 157 (4): 1333–1336); diabetes (Luhder, F. et al. (1998) supra). Indeed, induction of anti-tumor responses using tumor cell and peptide vaccines reveals that many anti-tumor responses involve anti-self reactivities (depigmentation observed in anti-CTLA-4+GM-CSF modified B16 melanoma in van Elsas et al. supra; depigmentation in Trp-2 vaccinated mice (Overwijk, W. et al. (1999) *Proc. Natl. Acad. Sci. USA.* 96: 2982–2987); autoimmune prostatitis evoked by TRAMP tumor cell vaccines (Hurwitz, A. (2000) supra), melanoma peptide antigen vaccination and vitilago observed in human clinical trials (Rosenberg, SA and White, Del. (1996) *J Immunother Emphasis Tumor Immunol* 19 (1): 81–4).

Therefore, it is possible to consider using anti-CTLA-4 blockade in conjunction with various self proteins in order to devise vaccination protocols to efficiently generate immune responses against these self proteins for disease treatment. For example, Alzheimers disease involves inap-

propriate accumulation of Aβ peptide in amyloid deposits in the brain; antibody responses against amyloid are able to clear these amyloid deposits (Schenk et al., (1999) Nature 400: 173–177).

Other self proteins may also be used as targets such as IgE for the treatment of allergy and asthma, and TNF for rhematoid arthritis. Finally, antibody responses to various hormones may be induced by the use of anti-CTLA-4 antibody. Neutralizing antibody responses to reproductive hormones may be used for contraception. Neutralizing antibody response to hormones and other soluble factors that are required for the growth of particular tumors may also be considered as possible vaccination targets.

Analogous methods as described above for the use of anti-CTLA-4 antibody can be used for induction of therapeutic autoimmune responses to treat patients having an inappropriate accumulation of other self-antigens, such as amyloid deposits, including Aβ in Alzheimer's disease, cytokines such as TNFα, and IgE.

2. Inactivating Immune Responses

Disorders caused by immune responses are called hypersensitivity diesease. Diseases caused by failure of self-tolerance and subsequent immune responses against self, or autologous, antigens are called autoimmune diseases. Hypersensitivity diseases can also result from uncontrolled or excessive responses against foreign antigens, such as microbes.

Although soluble antibodies to human CTLA-4 have been shown to promote the expansion and activation of T cells (i.e., where CTLA-4 function (e.g., binding to ligand) is inhibited; in this scenario the antibodies are antagonists to CTLA-4 function), increasing the valency of these same antibodies produces the opposite effect (where now, in contrast, the antibodies are acting as agonists of CTLA-4 to suppress the immune response) (see, e.g., Krummel and Allison, 1996, *J. Exp. Med.* 183, 2533–2540). For the purposes of inactivating antigen specific T cell responses, such as those that are the targets of pathogenic autoreactive T cells, the target antigen which is specific for these T cells (ie. antigen and/or MHC/antigen complexes) must be administered with the polyvalent form of anti-CTLA-4 antibody.

a. Inflammation

Inflammation represents the consequence of capillary dilation with accumulation of fluid and migration of phagocytic leukocytes, such as granulocytes and monocytes. Inflammation is important in defending a host against a variety of infections but can also have undesirable consequences in inflammatory disorders, such as anaphylactic shock, arthritis, gout and ischemia-reperfusion. Activated T-cells have an important modulatory role in inflammation, releasing interferon y and colony stimulating factors that in turn activate phagocytic leukocytes. The activated phagocytic leukocytes are induced to express a number of specific cells surface molecules termed homing receptors, which serve to attach the phagocytes to target endothelial cells. Inflammatory responses can be reduced or eliminated by treatment with the therapeutic agents of the present invention. For example, polyvalent preparations of antibodies against CTLA-4 block activation of activated T-cells, thereby preventing these cells from releasing molecules required for activation of phagocytic cell types.

b. Autoimmune Diseases

A further situation in which immune suppression is desirable is in treatment of autoimmune diseases such as insulin-dependent diabetes mellitus, multiple sclerosis, stiff man syndrome, rheumatoid arthritis, myasthenia gravis and lupus erythematosus. In these diseases, the body develops a cellular and/or humoral immune response against one of its own antigens leading to destruction of that antigen, and potentially crippling and/or fatal consequences. Activated T-cells are believed to play a major role in many autoimmune diseases such as diabetes mellitus. Autoimmune diseases are treated by administering one of the therapeutic agents of the invention that inhibits activation of T cells. Optionally, the autoantigen, or a fragment thereof, against which the autoimmune disease is targeted can be administered shortly before, concurrently with, or shortly after the immunosuppressive agent. In this manner, tolerance can be induced to the autoantigen under cover of the suppressive treatment, thereby obviating the need for continued immunosuppression. See, e.g., Cobbold et al., WO 90/15152 (1990).

C. Graft Versus Host Disease

A related use for the therapeutic agents of the present invention is in modulating the immune response involved in "graft versus host" disease (GVHD). GVHD is a potentially fatal disease that occurs when immunologically competent cells are transferred to an allogeneic recipient. In this situation, the donor's immunocompetent cells may attack tissues in the recipient. Tissues of the skin, gut epithelia and liver are frequent targets and may be destroyed during the course of GVHD. The disease presents an especially severe problem when immune tissue is being transplanted, such as in bone marrow transplantation; but less severe GVHD has also been reported in other cases as well, including heart and liver transplants. The therapeutic agents of the present invention are used to inhibit activation of donor leukocytes, thereby inhibiting their ability to lyse target cells in the host.

d. Transplant Rejection

Over recent years there has been a considerable improvement in the efficiency of surgical techniques for transplanting tissues and organs such as skin, kidney, liver, heart, lung, pancreas and bone marrow. Perhaps the principal outstanding problem is the lack of satisfactory agents for inducing immune-tolerance in the recipient to the transplanted allograft or organ. When allogeneic cells or organs are transplanted into a host (i.e. the donor and donee are different individual from the same species), the host immune system is likely to mount an immune response to foreign antigens in the transplant (host-versus-graft disease) leading to destruction of the transplanted tissue. $CD8^+$ cells, $CD4^+$ cells and monocytes are all involved in the rejection of transplant tissues. The therapeutic agents of the present invention are useful to inhibit T-cell mediated alloantigen-induced immune responses in the donee thereby preventing such cells from participating in the destruction of the transplanted tissue or organ.

B. Methods for Detecting/Measuring the Presence of CTLA-4 in a Sample

The invention further provides methods for detecting the presence of human CTLA-4 antigen in a sample, or measuring the amount of human CTLA-4 antigen, comprising contacting the sample, and a control sample, with a human monoclonal antibody, or an antigen binding portion thereof, which specifically binds to human CTLA-4, under conditions that allow for formation of a complex between the antibody or portion thereof and human CTLA-4. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of human CTLA-4 antigen in the sample.

C. Kits

Also within the scope of the invention are kits comprising the compositions (e.g., human sequence antibodies, human

antibodies, multispecific and bispecific molecules) of the invention and instructions for use. The kit can further contain a least one additional reagent, or one or more additional human antibodies of the invention (e.g., a human antibody having a complementary activity which binds to an epitope in CTLA-4 antigen distinct from the first human antibody). Kits typically include: a label indicating the intended use of the contents of the kit. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

EXAMPLES

Example 1

Generation of Cmu Targeted Mice Construction of a CMD Targeting Vector

The plasmid pICEmu contains an EcoRI/XhoI fragment of the murine Ig heavy chain locus, spanning the mu gene, that was obtained from a Balb/C genomic lambda phage library (Marcu et al. *Cell* 22: 187, 1980). This genomic fragment was subcloned into the XhoI/EcoRI sites of the plasmid pICEMI9H (Marsh et al.; Gene 32, 481–485, 1984). The heavy chain sequences included in pICEmu extend downstream of the EcoRI site located just 3' of the mu intronic enhancer, to the XhoI site located approximately 1 kb downstream of the last transmembrane exon of the mu gene; however, much of the mu switch repeat region has been deleted by passage in E. coli.

The targeting vector was constructed as follows (see FIG. 1). A 1.3 kb HindIII/SmaI fragment was excised from pICEmu and subcloned into HindIII/SmaI digested pBluescript (Stratagene, La Jolla, Calif.). This pICEmu fragment extends from the HindIII site located approximately 1 kb 5' of Cmu1 to the SmaI site located within Cmu1. The resulting plasmid was digested with SmaI/SpeI and the approximately 4 kb SmaI/XbaI fragment from pICEmu, extending from the Sma I site in Cmu1 3' to the XbaI site located just downstream of the last Cmu exon, was inserted. The resulting plasmid, pTAR1, was linearized at the SmaI site, and a neo expression cassette inserted. This cassette consists of the neo gene under the transcriptional control of the mouse phosphoglycerate kinase (pgk) promoter (XbaI/TaqI fragment; Adra et al. (1987) Gene 60: 65–74) and containing the pgk polyadenylation site (PvuII/HindIII fragment; Boer et al. (1990) Biochemical Genetics 28: 299–308). This cassette was obtained from the plasmid pKJ1 (described by Tybulewicz et al. (1991) *Cell* 65: 1153–1163) from which the neo cassette was excised as an EcoRI/HindIII fragment and subcloned into EcoRI/HindIII digested pGEM-7Zf (+) to generate pGEM-7 (KJ1). The neo cassette was excised from pGEM-7 (KJ1) by EcoRI/SalI digestion, blunt ended and subcloned into the SmaI site of the plasmid pTAR1, in the opposite orientation of the genomic Cmu sequences. The resulting plasmid was linearized with Not I, and a herpes simplex virus thymidine kinase (tk) cassette was inserted to allow for enrichment of ES clones bearing homologous recombinants, as described by Mansour et al. (1988) *Nature* 336: 348–352. This cassette consists of the coding sequences of the tk gene bracketed by the mouse pgk promoter and polyadenylation site, as described by Tybulewicz et al. (1991) Cell 65: 1153–1163. The resulting CMD targeting vector contains a total of approximately 5.3 kb of homology to the heavy chain locus and is designed to generate a mutant mu gene into which has been inserted a neo expression cassette in the unique SmaI site of the first Cmu exon. The targeting vector was linearized with PvuI, which cuts within plasmid sequences, prior to electroporation into ES cells.

Generation and Analysis of Targeted ES Cells

AB-1 ES cells (McMahon, A. P. and Bradley, A., (1990) Cell 62: 1073–1085) were grown on mitotically inactive SNL76/7 cell feeder layers (ibid.) essentially as described (Robertson, E. J. (1987) in Teratocarcinomas and Embryonic Stem Cells: a Practical Approach (E. J. Robertson, ed.) Oxford: IRL Press, p. 71–112). The linearized CMD targeting vector was electroporated into AB-1 cells by the methods described Hasty et al. (Hasty, P. R. et al. (1991) *Nature* 350: 243–246). Electroporated cells were plated into 100 mm dishes at a density of 1–2x106 cells/dish. After 24 hours, G418 (200 micrograms/ml of active component) and FIAU (5x10–7 M) were added to the medium, and drug-resistant clones were allowed to develop over 8–9 days. Clones were picked, trypsinized, divided into two portions, and further expanded. Half of the cells derived from each clone were then frozen and the other half analyzed for homologous recombination between vector and target sequences.

DNA analysis was carried out by Southern blot hybridization. DNA was isolated from the clones as described Laird et al. (Laird, P. W. et al., (1991) *Nucleic Acids Res.* 19: 4293). Isolated genomic DNA was digested with SpeI and probed with a 915 bp SacI fragment, probe A (FIG. 1), which hybridizes to a sequence between the mu intronic enhancer and the mu switch region. Probe A detects a 9.9 kb SpeI fragment from the wild type locus, and a diagnostic 7.6 kb band from a mu locus which has homologously recombined with the CMD targeting vector (the neo expression cassette contains a SpeI site). Of 1132 G418 and FIAU resistant clones screened by Southern blot analysis, 3 displayed the 7.6 kb Spe I band indicative of homologous recombination at the mu locus. These 3 clones were further digested with the enzymes BglI, BstXI, and EcoRI to verify that the vector integrated homologously into the mu gene. When hybridized with probe A, Southern blots of wild type DNA digested with BglI, BstXI, or EcoRI produce fragments of 15.7, 7.3, and 12.5 kb, respectively, whereas the presence of a targeted mu allele is indicated by fragments of 7.7, 6.6, and 14.3 kb, respectively. All 3 positive clones detected by the SpeI digest showed the expected BglI, BstXI, and EcoRI restriction fragments diagnostic of insertion of the neo cassette into the Cmu1 exon.

Generation of Mice Bearing the Mutated Mu Gene

The three targeted ES clones, designated number 264, 272, and 408, were thawed and injected into C57BL/6J blastocysts as described by Bradley (Bradley, A. (1987) in Teratocarcinomas and Embryonic Stem Cells: a Practical Approach. (E. J. Robertson, ed.) Oxford: IRL Press, p. 113–151). Injected blastocysts were transferred into the uteri of pseudopregnant females to generate chimeric mice representing a mixture of cells derived from the input ES cells and the host blastocyst. The extent of ES cell contribution to the chimera can be visually estimated by the amount of agouti coat coloration, derived from the ES cell line, on the black C57BL/6J background. Clones 272 and 408 produced only low percentage chimeras (i.e. low percentage of agouti pigmentation) but clone 264 produced high percentage male chimeras. These chimeras were bred with C57BL/6J females and agouti offspring were generated, indicative of germline transmission of the ES cell genome. Screening for the targeted mu gene was carried out by Southern blot analysis of BglI digested DNA from tail biopsies (as described above for analysis of ES cell DNA). Approximately 50% of the agouti offspring showed a hybridizing BglI band of 7.7 kb in addition to the wild type band of 15.7 kb, demonstrating a germline transmission of the targeted mu gene.

Analysis of Transgenic Mice for Functional Inactivation of Mu Gene

To determine whether the insertion of the neo cassette into Cmu1 has inactivated the Ig heavy chain gene, a clone 264 chimera was bred with a mouse homozygous for the JHD mutation, which inactivates heavy chain expression as a result of deletion of the JH gene segments (Chen et al., (1993) Immunol. 5: 647–656). Four agouti offspring were generated. Serum was obtained from these animals at the age of 1 month and assayed by ELISA for the presence of murine IgM. Two of the four offspring were completely lacking IgM (Table 1). Genotyping of the four animals by Southern blot analysis of DNA from tail biopsies by BglI digestion and hybridization with probe A (FIG. 1), and by StuI digestion and hybridization with a 475 bp EcoRI/StuI fragment (ibid.) demonstrated that the animals which fail to express serum IgM are those in which one allele of the heavy chain locus carries the JHD mutation, the other allele the Cmu1 mutation. Mice heterozygous for the JHD mutation display wild type levels of serum Ig. These data demonstrate that the Cmu1 mutation inactivates expression of the mu gene.

TABLE 1

Level of serum IgM, detected by ELISA, for mice carrying both the CMD and JHD mutations (CMD/JHD), for mice heterozygous for the JHD mutation (+/JHD), for wild type (129Sv x C57BL/6J)F1 mice (+/+), and for B cell deficient mice homozygous for the JHD mutation (JHD/JHD).

Mouse	Serum IgM (micrograms/ml)	Ig H chain genotype
42	<0.002	CMD/JHD
43	196	+/JHD
44	<0.002	CMD/JHD
45	174	+/JHD
129 x BL6 F1	153	+/+
JHD	<0.002	JHD/JHD

Example 2

Generation of HCo12 Transgenic Mice The HCo12 Human Heavy Chain Transgene

The HCo12 transgene was generated by coinjection of the 80 kb insert of pHC2 (Taylor et al., 1994, Int. Immunol., 6: 579–591) and the 25 kb insert of pVx6. The plasmid pVx6 was constructed as described below.

An 8.5 kb HindIII/SalI DNA fragment, comprising the germline human VH1–18 (DP-14) gene together with approximately 2.5 kb of 5' flanking, and 5 kb of 3' flanking genomic sequence was subcloned into the plasmid vector pSP72 (Promega, Madison, Wis.) to generate the plasmid p343.7.16. A 7 kb BamHI/HindIII DNA fragment, comprising the germline human VH5–51 (DP-73) gene together with approximately 5 kb of 5' flanking and 1 kb of 3' flanking genomic sequence, was cloned into the pBR322 based plasmid cloning vector pGP1f (Taylor et al. 1992, Nucleic Acids Res. 20: 6287–6295), to generate the plasmid p251f. A new cloning vector derived from pGP1f, pGP1k (Seq. ID #1), was digested with EcoRV/BamHI, and ligated to a 10 kb EcoRV/BamHI DNA fragment, comprising the germline human VH3–23 (DP47) gene together with approximately 4 kb of 5' flanking and 5 kb of 3' flanking genomic sequence. The resulting plasmid, p112.2RR.7, was digested with BamHI/SalI and ligated with the 7 kb purified BamHI/SalI insert of p251f. The resulting plasmid, pVx4, was digested with XhoI and ligated with the 8.5 kb XhoI/SalI insert of p343.7.16. A clone was obtained with the VH1-18 gene in the same orientation as the other two V genes. This clone, designated pVx6, was then digested with NotI and the purified 26 kb insert coinjected—together with the purified 80 kb NotI insert of pHC2 at a 1:1 molar ratio—into the pronuclei of one-half day (C57BL/6JxDBA/2J)F2 embryos as described by Hogan et al. (B. Hogan et al., Manipulating the Mouse Embryo, A Laboratory Manual, 2nd edition, 1994, Cold Spring Harbor Laboratory Press, Plainview N.Y.). Three independent lines of transgenic mice comprising sequences from both Vx6 and HC2 were established from mice that developed from the injected embryos. These lines are designated (HCo12)14881, (HCo12)15083, and (HCo12)15087. Each of the three lines were then bred with mice comprising the CMD mutation described in Example 1, the JKD mutation (Chen et al. 1993, EMBO J. 12: 811–820), and the (KCo5)9272 transgene (Fishwild et al. 1996, *Nature* Biotechnology 14: 845–851). The resulting mice express human heavy and kappa light chain transgenes in a background homozygous for disruption of the endogenous mouse heavy and kappa light chain loci.

Example 3

Generation of Human IgG Kappa Anti-Human CTLA-4 Monoclonal Antibodies

Cell Based Antigen

A DNA segment encoding a fusion protein comprising sequences from the human CTLA-4 and the murine CD3zeta genes was constructed by PCR amplification of cDNA clones together with bridging synthetic oligonucleotides. The encoded fusion protein contains the following sequences: i human CTLA-4 encoding amino acids 1–190 (containing the signal peptide, the extracellular domain of human CTLA-4 and the entirety of the presumed transmembrane sequence of human CTLA-4) and ii. murine CD3zeta from amino acid 52 to the carboxy terminus (Weissman et al. (1988) *Science* 239: 1018–1021). The amplified PCR product was cloned into a plasmid vector and the DNA sequence was determined. The cloned insert was then subcloned into the vector pBABE (which contains a gene encoding for puromycin resistance (Morganstern, JP and Land, H Nucl. Acids Res. 18: 3587–96 (1990)) to create pBABE-huCTLA-4/CD3z. pBABE-huCTLA-4/CD3z was transfected into the retroviral packaging line, ψ-2, and a pool of puromycin resistant cells were selected. These cells were co-cultured with the murine T cell hybridoma BW5147 (ATCC #TIB-47). After 2 days of co-culture the non-adherent BW5147 cells were removed and selected for resistance to puromycin. The puromycin resistant cell pool was subcloned by limiting dilution and tested for surface expression of human CTLA-4 by FACS. A clone expressing high levels of human CTLA4 at the cell surface was selected.

Soluble Antigen

Recombinant CTLA-4 fusion protein comprising the extracellular domain of human CTLA-4 was purchased from R&D Systems (Cat. #325-CT-200). Extracellular CTLA-4 fragment was prepared by proteolytic cleavage of the CTLA-4 fusion protein at a Factor Xa protease cleavage site located after the C-terminus of the CTLA-4 extracellular domain. Fusion protein was treated with Factor Xa at a ratio of 50:1 of fusion protein to Factor Xa, and the CTLA-4 fragment was isolated by passage over protein G-Sepharose and Mono Q HPLC. Fractions were tested for the presence of human CTLA-4 dimer were by SDS-PAGE and by binding to cells expressing mouse B7 molecules (LtkmB7.1: mouse Ltk(-) cells transfected with a mouse B7.1 cDNA clone expression vector). Positive fractions were pooled and dialyzed into PBS buffer.

Transgenic Mice

Two different strains of mice were used to generate CTLA-4 reactive monoclonal antibodies. Strain ((CMD)++; (JKD)++; (HCo7)11952+/++; (KCo5)9272+/++), and strain ((CMD)++; (JKD)++; (HCo12)15087+/++; (KCo5) 9272+/++). Each of these strains are homozygous for disruptions of the endogenous heavy chain (CMD) and kappa light chain (JKD) loci. Both strains also comprise a human kappa light chain transgene (KCo5), with individual animals either hemizygous or homozygous for insertion #11952. The two strains differ in the human heavy chain transgene used. Mice were hemizygous or homozygous for either the HCo7 or the HCo12 transgene. The CMD mutation is described above in Example 1. The generation of (HCo12)15087 mice is described in Example 2. The JKD mutation (Chen et al. 1993, EMBO J. 12: 811–820) and the (KCo5)9272 (Fishwild et al. 1996, *Nature Biotechnology* 14: 845–851) and (HCo7) 11952 mice, are described in U.S. Pat. No. 5,770,429 (Lonberg & Kay, Jun. 23, 1998).

Immunization

Transgenic mice were initially immunized i.p. with 1–3× 10^7 cells in PBS, or with 10–50 xug soluble fusion protein in adjuvant (either complete Freund's or Ribi). Immunized mice were subsequently boosted every 2 to 4 weeks i.p. with 1–3x10^7 cells in PBS. Animals were kept on protocol for 2 to 5 months. Prior to fusion, animals were boosted i.v. on days -3 and -2 with approximately 106 cells, or with 10–20 ug soluble antigen (fusion protein or fusion protein and extracellular fragment). Some animals also received fusion protein i.v. on day -4. Successful fusions resulting in CTLA-4 reactive IgG kappa monoclonal antibodies were obtained from mice immunized by a variety of different protocols, including cells only, soluble antigen only, and cell immunizations followed by soluble antigen given i.v. prior to fusion.

Fusions

Spleen cells were fused to mouse myeloma cells (line P3x63 Ag8.6.53, ATCC CRL 1580, or SP2/0-Ag14, ATCC CRL 1581) by standard procedures (Harlow and Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor N.Y.; Kennett et al. 1980, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analysis*. Plenum, N.Y.; Oi and Hertzenberg, 1980, *Immunoglobulin Producing Hybrid Cell Lines, in Selected Methods In Cellular Immunology*, ed. Mishell and Shiigi, pp. 357–372. Freeman, San Francisco; Halk, 1984, *Methods in Enzymology: Plant Molecular Biology*, ed. Weissbach and Weissbach, pp. 766–780, Academic Press, Orlando, Fla.). Cells were cultured in DMEM, 10% FBS, OPI (Sigma 0–5003), BME (Gibco 21985–023), 3% Origen Hybridoma Cloning Factor (Igen IG50–0615), and 5% P388d1 (ATCC TIB 63) conditioned media. HAT or HT supplement was added to the medium during initial growth and selection.

Hybridoma Screening

To identify hybridomas secreting human IgG kappa antibodies, ELISA plates (Nunc MaxiSorp) were coated overnight at 4° C. with 100 ul/well goat anti-human Fcgamma specific antibody (Jackson Immuno Research #109–006-098) at 1 ug/ml in PBS. Plates were washed and blocked with 100 ul/well PBS-Tween containing 1% BSA. Fifty ul cell culture supernatant was added followed by a 1–2 hour incubation. Plates were washed and then incubated for one hour with 100 ul/well goat anti-Kappa light chain conjugated to alkaline phosphatase or horseradish peroxidase (Sigma #A-3813, or #A-7164). Plates were washed three times in PBS-Tween between each step. An analogous assay was used to identify hybridomas that secrete human antibodies reactive with human CTLA-4. This assay was identical except that the ELISA plates were coated with recombinant CTLA-4 fusion protein instead of goat anti-human Fcgamma antibody.

Characterization of Monoclonal Antibodies

Seventy two hybridomas that were shown by ELISA to secrete human IgG kappa binding to human CTLA-4 were subcloned. Forty seven of these subclones were tested to determine if the secreted human antibodies bind to CTLA-4 expressing cells, and if the antibodies inhibit soluble CTLA-4 from binding to cells expressing B7. Binding was determined by flow cytometry. To measure inhibition, 50 microliters of each supernatant was incubated with 10^5 LtkmB7.1 cells and 25 ng recombinant CTLA-4 fusion protein. Mean channel fluorescence was then determined by flow cytometry. FIG. 2 shows inhibition of soluble CTLA-4 binding to cells expressing B7.1. Mean channel fluorescence (MCF) of LtkmB7.1 cells stained with recombinant human CTLA-4 fusion protein was determined in the presence of hybridoma supernatant. Hybridomas that secrete blocking antibodies resulted in lower MCF values. BNI3.1 (Cat.#34580D, Pharmingen, San Diego, Calif.) was used as a positive control mouse monoclonal antibody that blocks CTLA-4/B7 binding.

Approximately 40% of the hybridomas appear to strongly inhibit CTLA-4 binding to the B7 ligand.

Antibodies from clones 10D1.3, 4B6.12, and 11E8, were then assayed by BIAcore (Biacore AB, Uppsala, Sweden) to determine binding kinetics. Purified recombinant CTLA-4 extracellular fragment was coupled to the CM5 sensor chip@ 1200 RU. Binding was measured by adding antibody at concentrations of 0.25, 0.5, 1, 2.5, and 5 μg/ml at a flow rate of 5 ul/min. The binding curves were fit to a Langmuir binding model using BIAevaluation software (Biacore AB, Uppsala, Sweden). Antibodies were purified by protein-A Sepharose chromatography. Determined on and off rates are shown in Table 2:

TABLE 2

Kinetics of binding of human IgG kappa antibodies to recombinant CTLA-4 immobilized on a surface.

Hybridoma	ka (1/Ms)	kd (1/s)	Ka (1/M)
10D1.3	4.1×10^5	1.0×10^{-4}	4×10^9
4B6.12	5.1×10^5	1.3×10^{-4}	4×10^9
11E8	4.3×10^5	1.8×10^{-4}	2×10^9

Serial dilutions of 10 different human IgG kappa anti-human CTLA-4 monoclonal antibodies (3A4, 9A5, 2E2, 2E7, 4B6, 4E10, 5C4, 5G1, 11E8, and 11G1) were added to microtiter wells coated with recombinant CTLA-4 fusion protein. After a 2 hour incubation, biotinylated antibody 11E8 was added to each well at a concentration of 0.1 μg/ml. The samples were incubated for 30 minutes, washed, and bound antibody detected with alkaline phosphatase/streptavidin conjugate. The titrations are shown in FIG. 3. Antibody 11E8 binding was blocked by itself and 7 of the other human antibodies. However, binding was not blocked by antibodies 3A4 or 9A5. Reciprocal binding experiments showed that 11E8 binding did not block either 3A4 or 9A5 binding to CTLA-4.

DNA Sequence

RNA was extracted from approximately 2x10^6 cells of each subcloned hybridoma cell line and used to synthesize cDNA using reagents and protocols from Invitrogen (MicroFastTrack and cDNA Cycle: Cat. #L1310-01, and #K1520-

02, Invitrogen, Carlsbad, Calif.). Human immunoglobulin heavy and kappa light chain V region fragments were amplified by PCR using pfu polymerase (Stratagene, La Jolla, Calif.), degenerate FR1 primers and unique constant region primers. The resulting PCR fragments were cloned into the pCR-Blunt vector (Invitrogen, Carlsbad, Calif.) and the sequence of the insert determined. The preliminary sequences for the heavy and light chain fragment of hybridoma 10D1.3 are shown in FIG. **4**. The determined sequences for the heavy and light chain fragment of hybridoma 10D1.3 are shown in FIG. **5** through FIG. **8**.

bined antibody variable genes. The germline sequence is then used to fill in missing portions of the variable region. Heavy and light chain leader sequences are cleaved during protein maturation and do not contribute to the properties of the final antibody. For this reason it is not necessary to use the corresponding germline leader sequence for expression constructs. To add missing sequences, cloned cDNA sequences can be combined with synthetic oligonucleotides by ligation or PCR amplification. Alternatively, the entire variable region can be synthesized as a set of short, overlapping, oligonucleotides and combined by PCR ampli-

TABLE 3

CDR sequences of light and heavy chains for MAbs 10D1, 4B6, and 1E2.

Chain	HuMAb	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
Light	10D1	RASQSVGSSYLA	24	GAFSRAT	29	QQYGSSPWT	35
Chain	4B6	RASQSVSSSFLA	25	GASSRAT	30	QQYGSSPWT	35
	1E2	RASQGISSWLA	26	AASSLQS	31	QQYNSYPPT	36
Heavy	10D1	SYTMH	27	FISYDGNNKYYADSVKG	32	TGWLGPFDY	37
Chain	4B6	SYTMH	27	FISYDGSNKHYADSVKG	33	TGWLGPFDY	37
	1E2	SYGMH	28	VIWYDGSNKYYADSVKG	34	APNYIGAFDV	38

Example 4

Use of Partial Antibody Sequences to Express Intact Antibodies

Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complimentarily determining regions (CDR's). For this reason, the amino acid sequences within CDR's are more diverse between individual antibodies than sequences outside of CDR's. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (Jones et al. 1986, *Nature* 321, 522–525). Such framework sequences can be obtained from public DNA databases that include germline antibody gene sequences. These germline sequences will differ from mature antibody gene sequences because they will not include completely assembled variable genes, which are formed by V(D)J joining during B cell maturation. Germline gene sequences will also differ from the sequence of a high affinity secondary repertoire antibody at individual nucleotides because of somatic mutations. However, somatic mutations are not distributed evenly across the variable region. For example, somatic mutations are relatively infrequent in the amino-terminal portion of framework region **1** and in the carboxy-terminal portion of framework region **4**. Furthermore, many somatic mutations do not significantly alter the binding properties of the antibody. For this reason, it is not necessary to obtain the entire DNA sequence of a particular antibody in order to recreate an intact recombinant antibody having binding properties similar to those of the original antibody (see PCT/US99/05535 filed on Mar. 12, 1999, which is herein incorporated by reference for all purposes). Partial heavy and light chain sequence spanning the CDR regions is typically sufficient for this purpose. The partial sequence is used to determine which germline variable and joining gene segments contributed to the recombination to create an entirely synthetic variable region clone. This process has certain advantages such as elimination or inclusion of particular restriction sites, or optimization of particular codons.

The nucleotide sequences of heavy and light chain transcripts from a hybridomas are used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and kappa light chain sequences can differ from the natural sequences in three ways: strings of repeated nucleotide bases are interrupted to facilitate oligonucleotide synthesis and PCR amplification; optimal translation initiation sites are incorporated according to Kozak's rules (Kozak, 1991, J. Biol. Chem. 266, 19867–19870); and, HindIII sites are engineered upstream of the translation initiation sites.

For both the heavy and light chain variable regions, the optimized coding, and corresponding non-coding, strand sequences are broken down into 30–50 nucleotide segments such that the breaks between nucleotides for the coding strand sequence occur at approximately the midpoint of the corresponding non-coding oligonucleotide. Thus, for each chain, the oligonucleotides can be assemble into overlapping double stranded sets that completely span the desired sequence. These oligonucleotides are combined into pools that span segments of 150–400 nucleotides. The pools are then used as templates to produce PCR amplification products of 150–400 nucleotides. Typically, a single variable region oligonucleotide set will be broken down into two pools which are separately amplified to generate two overlapping PCR products. These overlapping products are then combined by PCR amplification to form the complete variable region. It may also be desirable to include an overlapping fragment of the heavy or light chain constant region (including the BbsI site of the kappa light chain, or the AgeI site if the gamma heavy chain) in the PCR amplification to generate fragments that can easily be cloned into the expression vector constructs.

The reconstructed heavy and light chain variable regions are then combined with cloned promoter, translation initiation, constant region, 3' untranslated, polyadenylation,

and transcription termination, sequences to form expression vector constructs. The heavy and light chain expression constructs can be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a host cell expressing both chains.

Plasmids for use in construction of expression vectors for human IgGk are described below. The plasmids were constructed so that PCR amplified V heavy and V kappa light chain cDNA sequences could be used to reconstruct complete heavy and light chain minigenes. These plasmids can be used to express completely human, or chimeric IgG1k or IgG4k antibodies. Similar plasmids can be constructed for expression of other heavy chain isotypes, or for expression of antibodies comprising lambda light chains.

The kappa light chain plasmid, pCK7-96 (SEQ ID NO:39), includes the kappa constant region and polyadenylation site, such that kappa sequences amplified with 5' primers that include HindIII sites upstream of the initiator methionine can be digested with HindIII and BbsI, and cloned into pCK7-96 digested with HindIII and BbsI to reconstruct a complete light chain coding sequence together with a polyadenylation site. This cassette can be isolated as a HindIII/NotI fragment and ligated to transcription promoter sequences to create a functional minigene for transfection into cells.

The gamma1 heavy chain plasmid, pCG7-96 (SEQ ID NO:40), includes the human gamma1 constant region and polyadenylation site, such that gamma sequences amplified with 5' primers that include HindIII sites upstream of the initiator methionine can be digested with HindIII and AgeI, and cloned into pCG7-96 digested with HindIII and AgeI to reconstruct a complete gamma1 heavy chain coding sequence together with a polyadenylation site. This cassette can be isolated as a HindIII/SalI fragment and ligated to transcription promoter sequences to create a functional minigene for transfection into cells.

The gamma4 heavy chain plasmid, pG4HE (SEQ ID NO:41), includes the human gamma4 constant region and polyadenylation site, such that gamma sequences amplified with 5' primers that include HindIII sites upstream of the initiator methionine can be digested with HindIII and AgeI, and cloned into pG4HE digested with HindIII and AgeI to reconstruct a complete gamma4 heavy chain coding sequence together with a polyadenylation site. This cassette can be isolated as a HindIII/EcoRI fragment and ligated to transcription promoter sequences to create a functional minigene for transfection into cells.

A number of different promoters (including but not limited to CMV, ubiquitin, SRalpha, and beta-actin) can be used to express the reconstructed heavy and light chain genes. For example the vector pcDNA3.1+(Invitrogen, Carlsbad, Calif.), can be cleaved with HindIII and either NotI, XhoI, or EcoRI, for ligation with either the kappa, gamma1, or gamma4 cassettes described above, to form expression vectors that can be directly transfected into mammalian cells.

Example 5

10D1 Binding to CTLA-4

A. 10D1Binding to purified recombinant human CTLA-4

Binding of 10D1 to purified recombinant human CTLA-4 was shown by ELISA using standard methods and procedures (FIG. **9** and FIG. **10**). Microtiter plates coated with purified CTLA-4 were incubated with varying concentration of 10D1, and then developed with goat anti-human IgG $F(ab')_2$ conjugated to alkaline phosphatase. The data demonstrate dose-dependent binding of 10D1 that is well fit to a 4-parameter curve (correlation coefficient is −1.0). The half-maximal binding at 15 ng/ml reflects the high binding capacity of 10D1 to CTLA-4. Saturation of binding was observed at approximately 0.1 μg/ml.

B. 10D.1 Binding to CTLA-4 expressed on the plasma membrane of T-cells

In order to demonstrate binding of 10D1 to CTLA-4 expressed on the plasma membrane of T-cells, the results in FIG. **10** from a flow cytometric assay are shown. The flow cytometric assay was used with a T-cell line transfected to express high levels of human CTLA-4 (designated 58αβCTLA-4/CD3zeta cells). Varying concentrations of fluoresceinated 10D1 (10D1-FITC) were incubated with 58αβCTLA-4 cells. The cell associated fluorescence was determined by flow cytometry. As seen with the purified CTLA-4, 10D1 bound to CTLA-4-expressing cells in a dose-dependent manner that was well fit to a 4-paramater equation (correlation coefficient is −0.999). The half-maximal binding was 190 ng/ml, and saturation was achieved at 2 μg/ml. 10D1 did not bind to any CTLA-4-negative cell lines tested, including SKBR-3, BT474 and MCF10A breast epithelial tumors and L540 Hodgkin's tumor cells, nor did it bind to cells expressing murine CTLA-4. These data indicate the specificity of 10D1 for human CTLA. However, 10D1 was shown to cross-react with macaque CTLA-4 (see below).

C. Cross-Reactivity of 10D1 with Normal Human Tissues

In this study, a fluoresceinated form of the test article (10D1-FITC) was used to evaluate binding. The objective of the study was to evaluate potential cross-reactivity of 10D1-FITC with cryosections of normal human tissues. No unanticipated cross-reactivity was observed.

The study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice (GLP) Regulations (21 CFR Part 58). The human tissue panel included all the tissue on the "suggested list of human tissues to be used for immunohistochemical investigations of cross reactivity" in Annex II of the EC CPMP Guideline III/5271/94, "Production and quality control of monoclonal antibodies" and all the tissues recommended in the 1997 US FDA/CBER "Points to Consider in the Manufacture and Testing of Monclonal Antibody Products for Human Use".

Using an indirect immunoperoxidase method, 10D1-FITC specifically stained positive control, human CTLA-4-expressing, 58αβCTLA4CD3zeta cells as well as positive control lymphocytes in human tonsil. 10D1-FITC reactivity was moderate to intense and two concentrations of antibody were examined (10 μg/ml and 2.5 μg/ml). In both positive control 58αβCTLA4CD3zeta and positive control human tonsillar lymphocytes, 10D1-FITC specifically stained discrete, round, granules at membrane and in the cytoplasm immediately below the membrane. Reactivity was observed with occasional follicular, interfollicular, and subepithelial lymphocytes. Less than 1–2% of all tonsillar lymphocytes were reactive with 10D1-FITC.

10D1-FITC did not react with negative control human brain (cerebellum). An isotype-matched negative control antibody (HulgG1-k-FITC) did not specifically bind to either the positive control human CTLA-4-expressing 58αβCTLA4CD3zeta or human tonsil; nor did it bind specifically to negative control human brain (cerebellum).

To determine cross-reactivity, 10D1-FITC was applied to a panel of normal human tissues at two concentrations (10 μg/ml and 2.5% g/ml). Specific 10D1-FITC reactivity was observed for lymphocytes in the tonsil (⅔ donors), submu-

cosal lymphoid nodule in the colon (gastrointestinal tract-colon [⅓ donors]), and blood smears (⅔ donors).

Immunoreactive cells were identified as lymphocytes based on typical morphology (round molecular cells with large nucleus: cytoplasm ratio and scant cytoplasm, lack of dendritic processes, 10–15 μm in diameter) and location within the tissues (e.g., typical location within lymphoid tissues). In the tonsils from all three donors (test tissues), lymphocytes, 10D1-FITC specifically stained discrete, round, granules at membrane and in the cytoplasm immediately below the membrane. Reactivity was observed with occasional follicular, interfollicular and subepithelial lymphocytes. Less than 1–2% of all tonsillar lymphcytes were reactive with 10D 1-FITC.

In ⅓ donors examined, 10D1-FITC also specifically stained discrete granules in occasional follicular and interfollicular lymphocytes located in submucosal lymphoid nodules in the colon (gastrointestinal tract-colon [large intestine]). Again, discrete membrane granules were stained.

In peripheral blood smears from two of the three donors examined, 10D 1-FITC specifically stained discrete granules approximately 1 μm in diameter associated with the membrane of rare lymphocytes. The granules were arranged in a ring or in a curved pattern. Less than 1–2% of all peripheral blood leukocytes were reactive with 10D1-FITC.

Table 4. Cross-Reactivity of MAb 10D1 With Normal Human Tissues

TABLE 4

Cross-Reactivity of MAb 10D1 With Normal Human Tissues

	Test Article 10D1-FITC		Negative Control Antibody HulgG1-k-FITC		Assay	
Tissue	10 μg/ml	2.5 μg/ml	10 μg/ml	2.5 μg/ml	Control*	β_2-microglobulin
Positive Control 58αβCTLA4CD3zeta cells	3–4+	2–4+	Neg	Neg	Neg	Pos
Positive Control Lymphocytes in human tonsil	2–3+	2–3+	Neg	Neg	Neg	Pos
Negative Control Human brain - cerebellum	Neg	Neg	Neg	Neg	Neg	Pos
Adrenal	Neg	Neg	Neg	Neg	Neg	Pos
Blood						Pos
Neutrophils	Neg	Neg	Neg	Neg	Neg	Pos
Lymphocytes	2+ (rare)	Neg	Neg	Neg	Neg	Pos
Eosinophils	Neg	Neg	Neg	Neg	Neg	Pos
Monocytes	Neg	Neg	Neg	Neg	Neg	Pos
Platelets	Neg	Neg	Neg	Neg	Neg	Pos
Blood Vessel (endothelium) Examined in all tissues	Detailed under individual tissues					
Bone Marrow	Neg	Neg	Neg	Neg	Neg	Pos
Brain - Cerebellum	Neg	Neg	Neg	Neg	Neg	Pos
Brain - Cerebrum (cortex)	Neg	Neg	Neg	Neg	Neg	Pos
Breast (mammary gland)	Neg	Neg	Neg	Neg	Neg	Pos
Eye	Neg	Neg	Neg	Neg	Neg	Pos
Gastrointestinal Tract - Colon (large intestine) Submucosal lymphoid nodule (occasional follicular and interfollicular lymphocytes)	2–3+	2–3+	Neg	Neg	Neg	Pos
Gastrointestinal Tract - Colon (large intestine) Other elements	Neg	Neg	Neg	Neg	Neg	Pos
Gastrointestinal Tract - Esophagus	Neg	Neg	Neg	Neg	Neg	Pos
Gastrointestinal Tract - Small intestine	Neg	Neg	Neg	Neg	Neg	Pos
Gastrointestinal Tract - Stomach	Neg	Neg	Neg	Neg	Neg	Pos
Heart	Neg	Neg	Neg	Neg	Neg	Pos
Kidney (glomerulus, tubule)	Neg	Neg	Neg	Neg	Neg	Pos
Liver	Neg	Neg	Neg	Neg	Neg	Pos
Lung	Neg	Neg	Neg	Neg	Neg	Pos
Lymph Node	Neg	Neg	Neg	Neg	Neg	Pos
Ovary	Neg	Neg	Neg	Neg	Neg	Pos
Fallopian Tube (oviduct)	Neg	Neg	Neg	Neg	Neg	Pos
Pancreas	Neg	Neg	Neg	Neg	Neg	Pos
Parathyroid	Neg	Neg	Neg	Neg	Neg	Pos
Peripheral Nerve	Neg	Neg	Neg	Neg	Neg	Pos
Pituitary	Neg	Neg	Neg	Neg	Neg	Pos
Placenta	Neg	Neg	Neg	Neg	Neg	Pos
Prostate	Neg	Neg	Neg	Neg	Neg	Pos
Salivary Gland	Neg	Neg	Neg	Neg	Neg	Pos
Skin	Neg	Neg	Neg	Neg	Neg	Pos

TABLE 4-continued

Cross-Reactivity of MAb 10D1 With Normal Human Tissues

Tissue	Test Article 10D1-FITC 10 μg/ml	Test Article 10D1-FITC 2.5 μg/ml	Negative Control Antibody HulgG1-k-FITC 10 μg/ml	Negative Control Antibody HulgG1-k-FITC 2.5 μg/ml	Assay Control*	Assay β_2-microglobulin
Spinal Cord	Neg	Neg	Neg	Neg	Neg	Pos
Spleen	Neg	Neg	Neg	Neg	Neg	Pos
Striated (Skeletal) Muscle	Neg	Neg	Neg	Neg	Neg	Pos
Testis	Neg	Neg	Neg	Neg	Neg	Pos
Thymus	Neg	Neg	Neg	Neg	Neg	Pos
Thyroid	Neg	Neg	Neg	Neg	Neg	Pos
Tonsil Lymphocytes (occasional follicular, Interfollicular and subepithelial lymphocytes)	2+	1–2+	Neg	Neg	Neg	Pos
Tonsil Other elements	Neg	Neg	Neg	Neg	Neg	Pos
Ureter	Neg	Neg	Neg	Neg	Neg	Pos
Urinary Bladder	Neg	Neg	Neg	Neg	Neg	Pos
Uterus - Body (endometrium)	Neg	Neg	Neg	Neg	Neg	Pos
Uterus - Cervix	Neg	Neg	Neg	Neg	Neg	Pos

*omission of test antibody

D. Specific Reactivity of 10D.1 with macaque CTLA-4

Specific reactivity with macaque CTLA-4 was demonstrated using T-cells transfected to express the macaque CTLA-4 at high levels (Table 5). These data suggest that the CTLA-4 epitope for 10D1 is conserved between macaque and humans, therefore macaque is a good model to evaluate in vivo safety of anti-CTLA4 HuMAb 10D1.

Table 5

TABLE 5

Species	reactivity of isotype control (MFI)	reactivity of 10D1 (MFI)
human CTLA4	3	662
macaque CTLA4	4	606
murine CTLA4 (negative control)	5	5

MAb 10D1 (10 μg/ml) was incubated with cell lines expressing recombinant CTLA-4 from various species, and detected by FITC-anti human IgG. The cell-associated fluorescence was determined by FACScan and reported as mean fluorescence intensity (MFI). These data show that MAb 10D1 reacts well with macaque and human CTLA-4, but not with murine CTLA-4.

Example 6

10D1 Blocking of CTLA-4 to B7 Ligands

In order to show that 10D1 binding to CTLA-4 blocks the interaction of CTLA-4 with CTLA-4 ligands, B7.1 and B7.2, competition assays were performed by flow cytometry (FIG. **11** and FIG. **12**). As shown in FIG. **11**, FITC-labeled human B7.2-Ig fusion protein was incubated with 58αβCTLA-4 T-cells and various concentrations of 10D1 MAb. In FIG. **12**, FITC-labeled CTLA-4-Ig fusion protein was incubated with murine B7.1 transfected cells and various concentrations of 10D1 MAb.

The competition assays demonstrate the ability of 10D1 to efficiently inhibit CTLA-4-B7 interactions at low concentrations (1–10 μg/ml). The effective concentration would likely be much lower under physiological conditions, which would have far lower concentrations of CTLA-4 and B7 molecules. Similar data was obtained using biotinylated reagents in ELISA assays.

These in vitro studies demonstrate that MAb 10D1 binds human CTLA-4 with high affinity and specificity and that binding of 10D1 abrogates interaction between B7 co-stimulatory molecules and CTLA-4. These data for 10D1 are consistent with the in vitro activity profiles for anti-murine CTLA-4 antibodies that have demonstrated efficacy in murine tumor models.

Example 6

Epitope Mapping of 10D.1

Competitive ELISAs were done with biotin labeled and unlabeled antibodies to determine CTLA-4 epitope specificity. Four anti-CTLA4 epitope binding groups were identified among the human antibodies, and an additional two epitopes were defined by the commercial murine monclonal antibodies BNI3 (Pharmingen, San Diego, Calif.), and 8H5 (Ancell Corp. Bayport, Mn). FIGS. **3**, and **13A–13G** show results of competitive binding assays that demonstrate differential competition among the antibodies for binding to CTLA-4. These results are summarized in Table 6.

Antibodies in anti-CTLA-4 epitope binding groups ***4a*** and ***4b*** have similar binding characteristics, and additionally are strong blockers of CTLA-4-Ig binding to cell surface expressed B7.1 (Table 6). For example, FIG. **3** shows results with biotin labeled 11E8 antibody and 10 unlabeled antibodies (3A4, 9A5, 2E2, 2E7, 4B6, 4E10, 5C4, 5G1, 11E8 and 11G1). Antibody 11E8 binding was blocked by itself and 7 of the other human antibodies in epitope groups ***4a*** and ***4b***. However, binding of 11E8 was not blocked by antibodies 3A4 or 9A5(epitope groups 1 and 2). Reciprocal binding experiments showed that 11E8 binding did not block either 9A5 or 3A4 binding to CTLA-4 (FIGS. **13A** and **13B**). Similar results are shown for epitope group ***4a*** antibodies 10D1 and murine antibody 147 (FIGS. **13D** and **13F**). Antibodies in epitope group ***4b*** (FIG. **13E**) are similar to group ***4a*** antibodies with the exception that the epitope ***4b*** antibodies compete with epitope group 2 antibodies in

reciprocal binding experiments (FIG. 13B). Human antibodies that belong to epitope groups **3**, ***4a*** and ***4b*** are effective blockers of CTLA-4/B7.1 binding (FIG. **3**, and Table 6).

TABLE 6

CTLA-4 MABs: Epitope and CTLA-4/B7.1 Blocking Properties

Epitope	Monoclonal Antibody	Competition for CTLA-4 Binding	Blocks binding of CTLA-4-Ig to B7.1 on Lik mB7.1
1	9A5	No competition from groups 3, 4a, 4b, 5, and 6 Weak Competition form group 2	No
2	3A4	One way competition from groups 1, 4b, 5 and 6	No
	1E2	No competition with 4a. Weak competition form group 3	
3	5A8	Competes with 4a and 4b. Some competition with 2. No competition form 1 and 5	Yes
4a	10D1	Cross competes with all members of 4b.	Yes
	147*	Competition from 6 (non-reciprocal)	
	11E8	No competition with 1, 2, and 5.	
	11G1	Weak competition with 3.	
	4E10		
	5C4		
	3F10		
4b	4B6	Cross competes with all members of 4a	Yes
	4A1	Competes with 2	
	2E2	Weak competition with 3.	
	2E7	No competition with 1, and 5.	
	2G1	Competition from 6 (non-reciprocal)	
5	BNI3**	Competes with 6, no competition with groups 1 to 4	Yes
6	8H5***	Competes with 5, no competition with groups 1 to 4 Competition with group 3 not tested	Yes

*Murine monoclonal antibody

**Available from Pharmingen, BNI3 Catalog # 34580 D, San Diego CA.

***Available from Ancell, ANC 152.2/8H5 Catalog # 359-020, Ancell Corp. Bayport, Mn.

Example 7

10D1 Binds to Human Activated T Cells

The ability of 10D1 antibody to bind to CTLA-4 expressed by normal human T cells was investigated by flow cytometric analysis of resting and activated T cells (FIG. **14**). Freshly isolated human peripheral blood mononuclear cells at 2×10^6/ml were incubated in the presence or absence of 2 ug/ml of the T-cell mitogen, phytohemagglutinin (PHA). After four days incubation, the cells were washed and stained with the following antibodies: 1) no antibody; 2) HuIgG1-FITC, a human IgG1 anti EGF receptor antibody; 3) 10D1-FITC, human IgG1 antiCTLA-4 antibody; and 4) 147-FITC-mouse anti-human CTLA-4 antibody. After incubation for 1 hr., cells were washed and stained with rabbit anti-FITC IgG followed by goat anti-rabbit-PE. Analysis was performed on lymphocytes gated by forward versus side scatter. As shown in FIG. **14**, resting lymphocytes do not bind 10D1 antibody, while PHA-activated T cells express low levels of CTLA-4 at the cell surface.

Example 8

10D1 Does not Mediate Complement-Dependent or Antibody-Dependent Lysis of Activated T-Cells

The ability of MAb 10D1 to mediate complement-dependent cellular cytotoxicity (CDCC) or antibody-dependent cellular cytotoxicity (ADCC) of CTLA-4 expressing cells was investigated.

For CDCC experiments, rabbit serun was used as a source of compliment, in order to provide optimal conditions for CDCC. Rabbit complement has been shown to be more effective in mediating CDCC with human IgG_1 than human complement=(Jurianz, Maslak et al. 1999). PHA-stimulated T-cells were labeled with ^{51}Cr and incubated with various concentrations of anti-CTLA4 MAb 10D1 or anti-CD3 MAb with or without rabbit serum as a source of complement. After a 1 hour incubation, the ^{51}Cr released by dying cells was determined using a gamma counter. Target cells incubated with 2% SDS served as 100% lysis controls. The anti-CTLA-4 MAb 10D1 did not mediate CDCC of the activated T-cells (FIG. **15**). Under the same conditions, the murine $IgG2_a$ anti-CD3 MAb led to significant CDCC. Both murine $IgG2_a$ and human IgG_1 efficiently fix rabbit complement; therefore these differences most likely reflect the greatly reduced expression of CTLA-4 as compared to CD3 on activated T-cells.

Similarly, no ADCC activity was observed for MAb 10D1 using autologous mononuclear cells as effector cells (FIG. **16**). PHA-stimulated T-cells were labeled with ^{51}Cr and incubated with various concentrations of anti-CTLA4 MAb 10D1 or anti-CD3 MAb and fresh autologous mononuclear cells. The effector to target cell ratio was 100:1. After a 4 hour incubation, the ^{51}Cr released by dying cells was determined using a gamma counter. Target cells incubated with 2% SDS served as 100% lysis controls. Although the anti-CD3 MAb is a murine $IgG2_a$, which can mediate efficient ADCC with human effector cells, only low levels of ADCC were observed. These data are consistent with the requirement of high levels of antigen expression on the surface of target cells for efficient ADCC. Since MAb 10D1 is a human IgG_1, an isotype generally capable of mediating CDCC and ADCC, the lack of these activities is likely due to the very low expression of CTLA-4 on activated T-cells. Furthermore, the observation of increased numbers of activated T-cells in the primate toxicology studies (see below) is consistent with the lack of ADCC and CDCC activity of activated T-cells by MAb 10D1 in vivo.

Example 9

10D1 preclinical toxicity studies in cynomolgus monkeys

Two independent toxicology studies of 10D1 antibody and macaques were performed. A total of eight monkeys were analyzed. Four monkeys (two males and two females) tolerated three bolus i.v. doses of 3 mg/Kg human anti-CTLA4, and four monkeys (two males and two females) tolerated three bolus i.v. doses of 10 mg/Kg human anti-CTLA4 without significant clinical, immunotoxicology, or histopathological findings.

A. 10D1 primate toxicology study (3.0 mg/Kg)

To investigate the effects of 10D1 in vivo, a primate toxicology study was performed with two macaques. In a multiple dose toxicity study of MAb 10D1, this antibody was administered via intravenous injection of macaques. The objective of this study was to determine the tolerability of MAb 10D1 in two monkeys given at a dose and schedule compatible with efficacious treatment in a murine tumor regression model and proposed dose in human clinical studies. Two female cynomolgus monkeys (Macaca fascicilaris) were treated with three intravenous bolus doses of 3.0 mg/Kg 10D1 on days 1, 4, and 7 to evaluate safety and T-cell activation in these animals. The animals were observed for any adverse reactions, weight loss/gain, and morbidity and mortality up to 14 days post administration of the first dose. Seven days after the last dose the animals were sacrificed and necropsied to examine their organs individually. Blood samples were collected before each dose and before necropsy for examination of T-cell populations and expression of activation markers by flow cytometry. Plasma was also collected from blood samples to determine 10D1 antibody levels and anti-10D1 antibody responses by ELISA.

The animals tolerated three doses of antibody 10D1 without any clinical symptoms during the treatment course. The weight of these animals did not change significantly. No gross findings were documented on 47 organs/tissues examined at necropsy for either animal.

Histopathology studies were performed at Redfield laboratories, Redfield, Ark. The results from these studies indicated that multiple doses of MAb 10D1 did not produce acute toxicity in any of the organs and tissues examined.

Pharmacokinetic analysis revealed the presence of significant levels (up to 97.3 μg/ml) of 10D1 MAb in the plasma of both monkeys (see Table 7). Plasma levels of 10D1 were determined by a competition assay with FITC-10D1 using flow cytometry and 58αβTCTLA-4 T-cells.

TABLE 7

	10D1 plasma levels	
Time point	Monkey #1	Monkey #2
Pre-1st dose	0.0 (μg/ml plasma)	0.0 (μg/ml plasma)
Day 4, pre-2nd dose	17.4 (μg/ml plasma)	43.6 (μg/ml plasma)
Day 7, pre-3rd dose	83.6 (μg/ml plasma)	97.3 (μg/ml plasma)
Day 14	90.2 (μg/ml plasma)	70.9 (μg/ml plasma)

Evaluation of the anti-10D 1-antibody response was performed by ELISA. No significant anti-10D1 response was observed in either animal during the course of study (FIG. **17**). Microtiter plates were coated with 10D1MAb (for IgM assay) or 10D1 $F(ab')_2$ (for IgG assay). Dilutions of plasma samples from various time points were incubated with the plates, and anti-10D1 antibodies were detected with either anti-IgM or IgG Fc-specific alkaline phosphatase reagents. IgM anti-10D1 antibodies appear to have developed by day 14, however, the titers are very low. IgM anti-10D1 ant5ibodies appear to have developed by day 14, however, the titers are very low. These data demonstrate that the monkeys did not develop anti-10D1 antibody responses after 3 doses of the antibody.

These data demonstrate that the animals did not develop a significant antibody response against MAb 10D1 during the course of this study.

Immunotoxicology was investigated by flow cytometric analysis of lymphocyte populations during the course of the study. The lymphocyte subsets examined included CD3 as a marker for total T-cells and CD20 as a marker for total B-cells. T-cells, were further subdivided for expression of CD4 (helper T-cell marker) and CD8 (cytotoxic T-cell marker), as well as for activation markers CD25, CD29, CD69 and HLA-DR. No remarkable changes in T-cell populations or expression of activation markers was noted. The results are summarized in Table 8 below.

TABLE 8

	Flow cytometric analysis of lymphocyte populations	
Time point	Monkey #1	Monkey #2
Pre-1st dose	% CD3 = 61, % CD20 = 16	% CD3 = 54, % CD20 = 22
	% CD4 = 43, % CD8 = 50	% CD4 = 59, % CD8 = 36
	% CD25 ≦ 1, % CD29 = 41	% CD25 ≦ 1, % CD29 = 29
	% CD69 = <1, % HLA-DR = 4	% CD69 ≦ 1, % HLA-DR = 1
Day 4, pre-2nd dose	% CD3 = 58, % CD20 = 13	% CD3 = 56, % CD20 = 16
	% CD4 = 38, % CD8 = 52	% CD4 = 62, % CD8 = 37
	% CD25 ≦ 1, % CD29 = 52	% CD25 ≦ 1, % CD29 = 36
	% CD69 ≦ 1, % HLA-DR = 2	% CD69 ≦ 1, % HLA-DR ≦ 1
Day 7, pre-3rd dose	% CD3 = 59, % CD20 = 15	% CD3 = 51, % CD20 = 17
	% CD4 = 47, % CD8 = 59	% CD4 = 51, % CD8 = 39
	% CD25 = 2, % CD29 = 44	% CD25 = 1, % CD29 = 39
	% CD69 = 1, % HLA-DR = 4	% CD69 = 1, % HLA-DR = 2
Day 14	% CD3 = 64, % CD20 = 14	% CD3 = 59, % CD20 = 20
	% CD4 = 49, % CD8 = 44	% CD4 = 60, % CD8 = 35
	% CD25 = 1, % CD29 = 44	% CD25 ≦ 1, % CD29 = 34
	% CD69 ≦ 1, % HLA-DR = 15	% CD69 ≦ 1, % HLA-DR = 1

Heparinized blood samples were analyzed fresh by flow cytometry using FITC- or PE-labeled anti-lymphocyte

reagents. % CD3 and % CD20 are based on a lymphocyte gate. The additional T-cell markers and activation markers are all based on CD3-positive cells. These data indicate that multiple doses of MAb 10D1 does not have a significant effect on B and T-cell populations or T-cell activation markers.

B. 10D1 Primate Toxicology Study (3.0 and 10.0 mg/Kg)

Six cynomolgus monkeys (four males and two females), experimentally non-naïve and weighing 2.4 to 3.8 kg at the outset of the study, were assigned to treatment groups as shown in Table 9 below.

TABLE 9

Group No.	Number of Males/Females	Dose Level (mg/kg)	Dose Vol. (ml/kg)	Dose Solution Conc. mg/ml)
1	2/0	3	0.6	5.0
2	2/2	10	2.0	5.0

Each animal received a dose of human anti-CTLA4 (5 mg/ml concentration) by intravenous injection (i.e., "slow-push" bolus injection) every three days for one week (i.e., on Days 1, 4 and 7). Detailed clinical observations were conducted at least twice daily ("cageside observations"), and a thorough physical examination was performed on each animal prior to the study and on Day 12. Body weights were measured weekly (prestudy and Days 7 and 14), and ophthalmoscopic examination was conducted on all animals prior to the study and on Day 12. Blood samples for evaluation of serum chemistry, hematology and coagulation parameters were collected from all animals prestudy and on Day 14. Additional samples for selected hematology parameters (total and differential white blood cells only) were collected prior to dosing on each dosing day (Days 1, 4, and 7). Urine samples for standard urinalysis were obtained by drainage from specially designed cage-pans prior to dosing and on Day 13. Blood samples were also collected prior to each dose (Days 1, 4 and 7) and prior to termination (Day 14) for various analyses conducted by Medarex. These included analysis of test article concentration (pharmacokinetics), determination of the presence of antibodies to the test article, and flow cytometry analysis. All animals were euthanized on Day 14, at which time, a complete gross necropsy was conducted, major organs were weighed, and a standard complete set of tissues was collected from each animal and processed for examination by light microscopy.

Intravenous administration of human anti-CTLA4 at dose levels of 3 mg/kg and 10 mg/kg given every three days for a total of three doses was very well tolerated by cynomolgus monkeys. There were no clinical signs of toxicity from the cageside observations and physical examinations, and no effects on body weight, ocular examination findings, clinical pathology parameters, gross necropsy findings, organ weights or tissue histomorphology.

The results of the analysis of test article concentration in serum samples (i.e., trough levels measured in samples obtained prior to dosing on Days 4 and 7, and prior to necropsy on Day 14) indicated dose-dependent exposure to the test article. On Day 7, predose mean concentrations were approximately 84 and 240 μg/ml for the 3- and 10-mg/kg dose groups, respectively.

A potential for accumulation of the test article in serum with the every-three-day dosing schedule in monkeys was evident from the difference between the Day 4 and Day 7 trough levels (i.e., means concentrations on Day 7 were approximately twice as high as on Day 4), as well as from the high residual levels on Day 14 (one week after the last dose), which were similar to the Day 7 trough levels. Evidence of antibody formation against the test article was detected in two of the six study animals (one from Group 1 and another from Group 2). In the former case, it appeared that the antibody response might have affected the clearance of the test article from circulation. Flow cytometric analysis of lymphocyte subsets revealed a modest increase in total CD3-positive cells between Days 1 and Day 14, which correlated with an increase in CD3/CD4-positive cells, and a respective decrease in CD3/CD8-positive cells (Group 2 only). The percentage of CD3 cells expressing CD29 and HLA-DR moderately increased over the course of the study, which was consistent with previous findings that anti-CTLA4 antibodies can enhance antigen-specific T-cells.

In conclusion, apart from the minor changes in circulating lymphocyte subpopulations, the highest dose level tested in this study (i.e., three doses of 10 mg/kg given at three-day intervals) was an absolute no-effect dose level in cynomolgus monkeys.

Example 10

A Phase I Human Clinical trial of MAb 10D1 in Prostate Cancer (MDXCTLA4-01) and Melanoma (MDXCTLA4-02)

MDXCTLA4-01 is an open-label study of anti-cytotoxic T-lymphocyte-associated antigen-4 (anti-CTLA4) monoclonal antibody 10D1 (MAb 10D1in patients with progressive, metastatic, hormone-refractory prostate cancer. Treatment is a single dose of MAb 10D1 that is administered intravenously, as an infusion, at a dosage of 3.0 mg/Kg.

The objectives of this trial are to determine if i. administration of MAb 10D1 causes nonspecific T-cell activation, ii. to establish a safety/tolerability profile for MAb 10D1 in these patients and, iii. to determine the pharmacokinetic profile of MAb 10D1 and assess the development of a host immune response to MAb 10D1. In addition the study will attempt to identify preliminary evidence of efficacy. The study is a multicenter, open-label study of a single dose of MAb 10D1 in 14 subjects. The study consists of four phases: Screening, Infusion, Post-infusion, and Follow-up (see Table 10 below).

TABLE 10

Phase	Screen	Infusion	Post-infusion											Follow-up
Time	days -14 to 0	-30 to 130 min	145 min	160 min	190 min	250 min	370 min	24 hrs	48 hrs	72 hrs	day 7	day 14	day 21	day 28	monthly

Patients with histologic diagnosis of primary adenocarcinoma of the prostate, and progressive metastatic carcinoma

of the prostate after androgen deprivation and at least one systemic non-hormonal manipulation, are being screened for participation in this study. Subjects must have progressive measurable disease, progressive PSA, PSA >5 ng/ml, testosterone <50 ng/dl, primary gonadal androgen suppression, life expectancy >12 weeks, and Karnofsky Performance Status ≧60%.

Subjects undergo physical examination, ECG, chest radiography, diagnostic imaging, and blood sampling for hematological, biochemical, and immune function assessments, and have vital signs monitored. Monthly telephone interviews are used to collect and record information on a subset of adverse events, including autoimmune adverse events after disease progression, until six months after treatment. PSA (decline, duration of decline, progression, time to progression) and disease response (complete, partial, stable, progressive) are monitored. Plasma concentrations of MAb 10D1 are being assessed immediately prior to, during, and up to two months after, infusion.

Data from four prostate cancer subjects that have been treated are shown in Table 11. No adverse events have been recorded. For all of the subjects treated, MAb 10D1 appears to be well tolerated.

Because of the importance of monitoring the immune status of patients in the trial and the specific goal of monitoring generalized effects on T cell activation by anti-CTLA-4 antibody, the entry criteria in this study included minimum levels of CD4 and CD8 T cells of ≧500/ml and ≧500/ml respectively. However, it was observed during the initial accrual in the study that prostate cancer patients have significantly reduced T cell numbers although CD4 and CD8 T cells are clearly present. Many patients were initially rejected based on the above entry criteria (see Table 11). The apparent reduced T cell counts observed is a previously undocumented observation in prostate cancer patients that may have relevance in treatments involving cancer vaccination in these patients. Subsequent to these observations, the entry criteria were amended to include patients having CD4 and CD8 count of ≧300/ml and ≧200/ml respectively.

In order to evaluate whether administration of MAb 10D1 can induce undesirable non-specific T cell activation, peripheral blood lymphocytes from the prostate cancer subjects were analyzed by flow cytometry for each of the following markers: CD4, CD8, CD25, CD44, CD69 and HLA-DR. Blood samples were taken at time points indicated in Table 10. No significant change in the frequency of any of these markers was observed during the course of the treatment for each of the prostate cancer subjects treated thus far. An example of this analysis is shown in Table 12 which shows the frequency of CD4, CD25,CD69-positive cells and CD8, CD25,CD69-positive cells at times prior to, during, and subsequent to MAb 10D1 administration in two of the subjects. These data demonstrate that MAb 10D1 does not result in non-specific T cell activation.

TABLE 12

Flow cytometric analysis of T cell activation markers in prostate cancer subjects treated with 3.0 mg/Kg MAb 10D1.

Patient Number	Time Point	CD(4 + 25 + 69) %	CD(8 + 25 + 69) %
3	Screen	1.7	0.8
3	-30 MIN (Pre-Infusion)	2.6	0.8

TABLE 12-continued

Flow cytometric analysis of T cell activation markers in prostate cancer subjects treated with 3.0 mg/Kg MAb 10D1.

Patient Number	Time Point	CD(4 + 25 + 69) %	CD(8 + 25 + 69) %
3	40 MIN	2.5	0.7
3	130 MIN	1.9	0.9
3	145 MIN	1.7	0.5
3	160 MIN	1.7	1
3	190 MIN	1.5	1.5
3	250 MIN	2.1	1.2
3	370 MIN	1.3	0.9
3	24 HR	1.6	1.6
3	48 HR	2.7	3
3	72 HR	0.9	0.5
3	Day 7	0.9	0.1
3	Day 14	0.4	0.5
3	Day 21	2.3	1.9
4	Screen	1.4	0.8
4	-30 MIN (Pre-Infusion)	0.5	0.3
4	40 MIN	0.3	0.1
4	130 MIN	0.3	0.1
4	145 MIN	0.4	0.2
4	160 MIN	0.2	0.2
4	190 MIN	0.8	0.3
4	250 MIN	0.1	0
4	370 MIN	0.3	0.1
4	24 HR	0.2	0.3
4	48 HR	0.4	0.6
4	72 HR	0.8	0.3
4	Day 7	1	0.7
4	Day 14	1.1	0.8

A second clinical trial (MDXCTLA4-02) using MAb 10D1 in subjects with Stage IV malignant melanoma has also been initiated. A single dose of MAb 10D1 will be administered intravenously, as an infusion, at a dosage of 3.0 mg/Kg. This study also consists of four phases (Screening, Infusion, Post-Infusion and Follow-up) as described in Table 9, above.

The goals of this study are as those regarding the above-described study in prostate cancers as well as to specifically establish a safety/tolerability profile for MAb 10D1 in patients with Stage IV malignant melanoma. One patient has been treated in this study (see Table 13). As in the prostate cancer study, MAb 10D1 appears to be well tolerated. Flow cytometric analysis of T cell activation markers in this subject, analogous to that performed for the prostate tumor trial, also showed no evidence of non-specific T cell activation.

SEQ ID NO:1 pGP1k

```
AATTAGCGGC CGCTGTCGAC AAGCTTCGAA TTCAGTATCG ATGTGGGGTA   50
CCTACTGTCC CGGGATTGCG GATCCGCGAT GATATCGTTG ATCCTCGAGT  100
GCGGCCGCAG TATGCAAAAA AAAGCCCGCT CATTAGGCGG GCTCTTGGCA  150
GAACATATCC ATCGCGTCCG CCATCTCCAG CAGCCGCACG CGGCGCATCT  200
CGGGCAGCGT TGGGTCCTGG CCACGGGTGC GCATGATCGT GCTCCTGTCG  250
TTGAGGACCC GGCTAGGCTG GCGGGGTTGC CTTACTGGTT AGCAGAATGA  300
ATCACCGATA CGCGAGCGAA CGTGAAGCGA CTGCTGCTGC AAAACGTCTG  350
CGACCTGAGC AACAACATGA ATGGTCTTCG GTTTCCGTGT TTCGTAAAGT  400
CTGGAAACGC GGAAGTCAGC GCCCTGCACC ATTATGTTCC GGATCTGCAT  450
CGCAGGATGC TGCTGGCTAC CCTGTGGAAC ACCTACATCT GTATTAACGA  500
AGCGCTGGCA TTGACCCTGA GTGATTTTTC TCTGGTCCCG CCGCATCCAT  550
ACCGCCAGTT GTTTACCCTC ACAACGTTCC AGTAACCGGG CATGTTCATC  600
ATCAGTAACC CGTATCGTGA GCATCCTCTC TCGTTTCATC GGTATCATTA  650
CCCCCATGAA CAGAAATTCC CCCTTACACG GAGGCATCAA GTGACCAAAC  700
AGGAAAAAAC CGCCCTTAAC ATGGCCCGCT TTATCAGAAG CCAGACATTA  750
ACGCTTCTGG AGAAACTCAA CGAGCTGGAC GCGGATGAAC AGGCAGACAT  800
CTGTGAATCG CTTCACGACC ACGCTGATGA GCTTTACCGC AGCTGCCTCG  850
CGCGTTTCGG TGATGACGGT GAAAACCTCT GACACATGCA GCTCCCGGAG  900
ACGGTCACAG CTTGTCTGTA AGCGGATGCC GGGAGCAGAC AAGCCCGTCA  950
GGGCGCGTCA GCGGGTGTTG GCGGGTGTCG GGGCGCAGCC ATGACCCAGT 1000
CACGTAGCGA TAGCGGAGTG TATACTGGCT TAACTATGCG GCATCAGAGC 1050
AGATTGTACT GAGAGTGCAC CATATGCGGT GTGAAATACC GCACAGATGC 1100
GTAAGGAGAA AATACCGCAT CAGGCGCTCT TCCGCTTCCT CGCTCACTGA 1150
CTCGCTGCGC TCGGTCGTTC GGCTGCGGCG AGCGGTATCA GCTCACTCAA 1200
AGGCGGTAAT ACGGTTATCC ACAGAATCAG GGGATAACGC AGGAAAGAAC 1250
ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG AACCGTAAAA AGGCCGCGTT 1300
GCTGGCGTTT TTCCATAGGC TCCGCCCCCC TGACGAGCAT CACAAAAATC 1350
GACGCTCAAG TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG 1400
GCGTTTCCCC CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC CGACCCTGCC 1450
GCTTACCGGA TACCTGTCCG CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT 1500
CTCATAGCTC ACGCTGTAGG TATCTCAGTT CGGTGTAGGT CGTTCGCTCC 1550
AAGCTGGGCT GTGTGCACGA ACCCCCCGTT CAGCCCGACC GCTGCGCCTT 1600
ATCCGGTAAC TATCGTCTTG AGTCCAACCC GGTAAGACAC GACTTATCGC 1650
CACTGGCAGC AGCCAGGCGC GCCTTGGCCT AAGAGGCCAC TGGTAACAGG 1700
ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG 1750
GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC 1800
TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA 1850
CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC 1900
GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT 1950
CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA 2000
```

-continued

TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT 2050

TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT 2100

GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC 2150

ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT 2200

ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG 2250

CTCCAGATTT ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA 2300

AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA ATTGTTGCCG 2350

GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG 2400

CCATTGCTGC AGGCATCGTG GTGTCACGCT CGTCGTTTGG TATGGCTTCA 2450

TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT 2500

GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA 2550

AGTTGGCCGC AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT 2600

CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC 2650

AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC 2700

CGGCGTCAAC ACGGGATAAT ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG 2750

CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC 2800

GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT 2850

CAGCATCTTT TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG 2900

CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT 2950

CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC 3000

TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG 3050

GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT 3100

TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG AGGCCCTTTC 3150

GTCTTCAAG 3159

pCK7-96 (Nucleotide residues 3376 to 3881)(SEQ ID NO:39)

AGGAGAATGAATAAATAAAGTGAATCTTTTGCACCTGTGGTTTCTCTCTTTCCTCAATTTAATAATTATT

ATCTGTTGTTTACCAACTACTCAATTTCTCTTATAAGGGACTAAATATGTAGTCATCCTAAGGCGCATA

ACCATTTATAAAAATCATCCTTCATTCTATTTTACCCTATCATTCCTCTGCAAGACAGTCCTCCCTCAAA

CCCACAAGCCTTCTGTCCTCACAGTCCCCTGGGCCATGGATCCTCACATCCCAATCCGCGGCCGCAATT

CGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAG

CCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCT

CACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGA

GAGGCGGTTTGCGTATTGGGCGC

pCG7-96 (SEQ ID NO:40)

GAACTCGAGCAGCTGAAGCTTTCTGGGGCAGGCCAGGCCTGACCTTGGCTTTGGGGCAGGGAGGGGGCTAAGGTG

AGGCAGGTGGCGCCAGCCAGGTGCACACCCAATGCCCATGAGCCCAGACACTGGACGCTGAACCTCGCGGACAGT

TAAGAACCCAGGGGCCTCTGCGCCCTGGGCCCAGCTCTGTCCCACACCGCGGTCACATGGCACCACCTCTCTTGC

AGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCT

GGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGT

GCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG

-continued

CTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGGTGA

GAGGCCAGCACAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCTATG

CAGCCCCAGTCCAGGGCAGCAAGGCAGGCCCCGTCTGCCTCTTCACCCGGAGGCCTCTGCCCGCCCCACTCATGC

TCAGGGAGAGGGTCTTCTGGCTTTTTCCCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCAGGCCCTG

CACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACCCTGCCCCTGACCTAAGCC

CACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCGGACACCTTCTCTCCTCCCAGATTCCAGTAACTCCCAATC

TTCTCTCTGCAGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGGTAAGCCAGCCCAGGCCT

CGCCCTCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGCTGAC

ACGTCCACCTCCATCTCTTCCTCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCC

AAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG

GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC

AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG

GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGTGGGACCCGTGGGGTGCGA

GGGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTGCCCTGAGAGTGACCGCTGTACCAACCTCTGTCCCTAC

AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCT

GACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA

CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAG

CAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAG

CCTCTCCCTGTCTCCGGGTAAATGAGTGCGACGGCCGGCAAGCCCCCGCTCCCCGGGCTCTCGCGGTCGCACGAG

GATGCTTGGCACGTACCCCCTGTACATACTTCCCGGGCGCCCAGCATGGAAATAAAGCACCCAGCGCTGCCCTGG

GCCCCTGCGAGACTGTGATGGTTCTTTCCACGGGTCAGGCCGAGTCTGAGGCCTGAGTGGCATGAGGGAGGCAGA

GCGGGTCCCACTGTCCCCACACTGGCCCAGGCTGTGCAGGTGTGCCTGGGCCCCCTAGGGTGGGGCTCAGCCAGG

GGCTGCCCTCGGCAGGGTGGGGGATTTGCCAGCGTGGCCCTCCCTCCAGCAGCACCTGCCCTGGGCTGGGCCACG

GGAAGCCCTAGGAGCCCCTGGGGACAGACACACAGCCCCTGCCTCTGTAGGAGACTGTCCTGTTCTGTGAGCGCC

CCTGTCCTCCCGACCTCCATGCCCACTCGGGGGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGC

TCGAATTCATCGATGATATCAGATCTGCCGGTCTCCCTATAGTGAGTCGTATTAATTTCGATAAGCCAGGTTAAC

CTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACT

GACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACA

GAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCG

TTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA

AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTG

CCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTAT

CTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCC

TTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAC

AGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGA

AGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGC

AAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAA

GAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATG

AGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATAT

GAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCA

TCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCA

-continued

ATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGC

AGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCG

CCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCT

TCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC

TTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAAT

pG4HE (SEQ ID NO:41)

GAACTCGAGCAGCTGAAGCTTTCTGGGGCAGGCCGGGCCTGACTTTGGCTGGGGGCAGGGAGGGGGCTAAGGTGA

CGCAGGTGGCGCCAGCCAGGTGCACACCCAATGCCCATGAGCCCAGACACTGGACCCTGCATGGACCATCGCGGA

TAGACAAGAACCGAGGGGCCTCTGCGCCCTGGGCCCAGCTCTGTCCCACACCGCGGTCACATGGCACCACCTCTC

TTGCAGCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCG

CCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCG

GCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCA

GCAGCTTGGGCACGAAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTG

GTGAGAGGCCAGCACAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCCCTCCTGCCTGGACGCACCCCGGC

TGTGCAGCCCCAGCCCAGGGCAGCAAGGCATGCCCCATCTGTCTCCTCACCCGGAGGCCTCTGACCACCCCACTC

ATGCTCAGGGAGAGGGTCTTCTGGATTTTTCCACCAGGCTCCGGGCAGCCACAGGCTGGATGCCCCTACCCCAGG

CCCTGCGCATACAGGGGCAGGTGCTGCGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACCCTGCCCCTGACCT

AAGCCCACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCAGACACCTTCTCTCCTCCCAGATCTGAGTAACTCC

CAATCTTCTCTCTGCAGAGTCCAAATATGGTCCCCCATGCCCATCATGCCCAGGTAAGCCAACCCAGGCCTCGCC

CTCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGCTGACGCAT

CCACCTCCATCTCTTCCTCAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGG

ACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCC

AGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCA

CGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCT

CCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGTGGGACCCACGGGGTGCGAGGGC

CACATGGACAGAGGTCAGCTCGGCCCACCCTCTGCCCTGGGAGTGACCGCTGTGCCAACCTCTGTCCCTACAGGG

CAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACC

TGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC

AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAGCAGG

TGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTC

TCCCTGTCTCTGGGTAAATGAGTGCCAGGGCCGGCAAGCCCCCGCTCCCCGGGCTCTCGGGGTCGCGCGAGGATG

CTTGGCACGTACCCCGTCTACATACTTCCCAGGCACCCAGCATGGAAATAAAGCACCCACCACTGCCCTGGGCCC

CTGTGAGACTGTGATGGTTCTTTCCACGGGTCAGGCCGAGTCTGAGGCCTGAGTGACATGAGGGAGGCAGAGCGG

GTCCCACTGTCCCCACACTGGCCCAGGCTGTGCAGGTGTGCCTGGGCCACCTAGGGTGGGGCTCAGCCAGGGGCT

GCCCTCGGCAGGGTGGGGGATTTGCCAGCGTGGCCCTCCCTCCAGCAGCAGCTGCCCTGGGCTGGGCCACGGGAA

GCCCTAGGAGCCCCTGGGGACAGACACACAGCCCCTGCCTCTGTAGGAGACTGTCCTGTCCTGTGAGCGCCCTGT

CCTCCGACCCCCCATGCCCACTCGGGGGGATCCCCGGGTACCGAGCTCGAATTCATCGATGATATCAGATCTGCC

GGTCTCCCTATAGTGAGTCGTATTAATTTCGATAAGCCAGGTTAACCTGCATTAATGAATCGGCCAACGCGCGGG

GAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCG

GCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACAT

-continued

GTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCC

CCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGC

GTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCT

CCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAA

GCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAA

CCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGG

TGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCT

GAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTT

TTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTC

TGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGAT

CCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATG

CTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTA

GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGC

TCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTC

CATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGC

CATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAG

GCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAA

GTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATG

CTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCC

GGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGG

GCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTC

AGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAG

GGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCT

CATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGT

GCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCG

TCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTA

AGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTA

TGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGGACATATTGTCGTTAGAACGCGGCTACAATTAATA

CATAACCTTATGTATCATACACATACGATTTAGGTGACACTATA

10D1 VH(SEQ ID NO:16)

CAGGTGCAGC TGGTGGAGTC TGGGGGAGGC GTGGTCCAGC CTGGGAGGTC 50

CCTGAGACTC TCCTGTGCAG CCTCTGGATT CACCTTCAGT AGCTATACTA 100

TGCACTGGGT CCGCCAGGCT CCAGGCAAGG GGCTGGAGTG GGTGACATTT 150

ATATCATATG ATGGAAACAA TAAATACTAC GCAGACTCCG TGAAGGGCCG 200

ATTCACCATC TCCAGAGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA 250

ACAGCCTGAG AGCTGAGGAC ACGGCTATAT ATTACTGTGC GAGGACCGGC 300

TGGCTGGGGC CCTTTGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC 350

CTCAG

10D1 VK(SEQ ID NO:6)

GAAATTGTGT TGACGCAGTC TCCAGGCACC CTGTCTTTGT CTCCAGGGGA 50

AAGAGCCACC CTCTCCTGCA GGGCCAGTCA GAGTGTTGGC AGCAGCTACT 100

-continued

TAGCCTGGTA CCAGCAGAAA CCTGGCCAGG CTCCCAGGCT CCTCATCTAT 150

GGTGCATTCA GCAGGGCCAC TGGCATCCCA GACAGGTTCA GTGGCAGTGG 200

GTCTGGGACA GACTTCACTC TCACCATCAG CAGACTGGAG CCTGAAGATT 250

TTGCAGTGTA TTACTGTCAG CAGTATGGTA GCTCACCGTG GACGTTCGGC 300

CAAGGGACCA AGGTGGAAAT CAAAC 325

4B6 VH(SEQ ID NO:18)

CAGGTGCAGC TGGTGGAGTC TGGGGGAGGC GTGGTCCAGC CTGGGAGGTC 50

CCTGAGACTC TCCTGTGCAG CCTCTGGATT CACCTTCAGT AGCTATACTA 100

TGCACTGGGT CCGCCAGGCT CCAGGCAAGG GGCTGGAGTG GGTGACATTT 150

ATATCATATG ATGGAAGCAA TAAACACTAC GCAGACTCCG TGAAGGGCCG 200

ATTCACCGTC TCCAGAGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA 250

ACAGCCTGAG AGCTGAGGAC ACGGCTATAT ATTACTGTGC GAGGACCGGC 300

TGGCTGGGGC CCTTTGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC 350

CTCAG

4B6 VK(SEQ ID NO:8)

GAAATTGTGT TGACGCAGTC TCCAGGCACC CTGTCTTTGT CTCCAGGGGA 50

AAGAGCCACC CTCTCCTGCA GGGCCAGTCA GAGTGTTAGC AGCAGCTTCT 100

TAGCCTGGTA CCAGCAGAAA CCTGGCCAGG CTCCCAGGCT CCTCATCTAT 150

GGTGCATCCA GCAGGGCCAC TGGCATCCCA GACAGGTTCA GTGGCAGTGG 200

GTCTGGGACA GACTTCACTC TCACCATCAG CAGACTGGAG CCTGAAGATT 250

TTGCAGTGTA TTACTGTCAG CAGTATGGTA GCTCACCGTG GACGTTCGGC 300

CAAGGGACCA AGGTGGAAAT CAAAC 325

1E2 VH(SEQ ID NO:22)

CAGGTGCAGC TGGTGGAGTC TGGGGGAGGC GTGGTCCAGC CTGGGAGGTC 50

CCTGAGACTC TCCTGTGCAG CGTCTGGATT CACCTTCAGT AGCTATGGCA 100

TGCACTGGGT CCGCCAGGCT CCAGGCAAGG GGCTGGAGTG GGTGGCAGTT 150

ATATGGTATG ATGGAAGTAA TAAATACTAT GCAGACTCCG TGAAGGGCCG 200

ATTCACCATC TCCAGAGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA 250

ACAGCCTGAG AGCCGAGGAC ACGGCTGTGT TTTACTGTGC GAGAGCTCCC 300

AATTATATTG GTGCTTTTGA TGTCTGGGGC CAAGGGACAA TGGTCACCGT 350

CTCTTCAG

1E2 VK(SEQ ID NO:12)

GACATCCAGA TGACCCAGTC TCCATCCTCA CTGTCTGCAT CTGTAGGAGA 50

CAGAGTCACC ATCACTTGTC GGGCGAGTCA GGGTATTAGC AGCTGGTTAG 100

CCTGGTATCA GCAGAAACCA GAGAAAGCCC CTAAGTCCCT GATCTATGCT 150

GCATCCAGTT TGCAAAGTGG GGTCCCATCA AGGTTCAGCG GCAGTGGATC 200

TGGGACAGAT TTCACTCTCA CCATCAGCAG CCTGCAGCCT GAAGATTTTG 250

CAACTTATTA CTGCCAACAG TATAATAGTT ACCCTCCGAC GTTCGGCCAA 300

GGGACCAAGG TGGAAATCAA AC 322

TABLE 11

Study No. MDXCTLA4-01
Selected Lab Values Summary

Screen no.	Subject no.	Initials	Amendment #	Day	Date	PSA	Platelets	WBC	Neuts		Lymphs		Monos		Eos		CD4/	CD8/	ESR	Hgb	Hcrit
						ng/ml	$\times 10^3$/ul	$\times 10^3$/ul	%	$\times 10^3$/ul	%	$\times 10^3$/ul	%	$\times 10^3$/ul	%	$\times 10^3$/ul	ul	ul	mm/hr	g/dl	%
02001	001	JGR		Scr		144.80	263	8.12	73.00	5.90	18.00	1.47	5.60	0.46	1.80	0.15	670	367	71	10.4	30
02001	001	JGR		0		185.20	267	5.74	66.00	3.79	22.00	1.32	6.60	0.38	3.10	0.18	704	376		10.6	32
02001	001	JGR		1			259	6.31	69.00	4.38	20.00	1.29	8.70	0.55	0.90	0.00	A	A		9.5	30
02001	001	JGR		2			240	6.59	70.00	4.66	19.00	1.31	6.70	0.44	1.80	0.12	556	303		9.5	28
02001	001	JGR		3			270	6.53	71.00	4.63	21.00	1.36	5.50	0.36	2.20	0.14	608	254		9.3	28
02001	001	JGR		7		257.40	299	6.70	68.00	4.56	23.00	1.53	6.00	0.40	2.50	0.17	A	A		9.5	28
02001	001	JGR		14		332.30	308	6.87	71.90	7.94	21.20	1.39	5.21	0.36	1.90	0.13	A	A		8.8	25
02001	001	JGR		21			286	9.72	74.00	7.20	19.70	1.91	4.80	0.46	1.00	0.10	A	A		9.1	28
02001	001	JGR		28		351.00	304	5.38	63.00	3.40	26.00	1.44	5.80	0.31	2.90	0.16				8.7	25
01002		JWF		Scr		28.30	271	11.60	75.40	8.75	13.60	1.58	5.70	0.66	4.60	0.53	399	189	41	13.9	37
01003		MZB		Scr		12.70	178	5.49	69.00	3.79	19.60	1.08	6.30	0.35	2.70	0.24	325	168	19	12.7	36
01004		TEQ		Scr		1459.00	264	6.26	75.10	4.70	14.40	0.90	7.70	0.48	2.40	0.15	365	129	61	12.8	36
01005		WMN		Scr		192.40	212	6.85	73.70	5.05	17.40	1.20	6.20	0.43	2.20	0.15	483	217			
01006		MRS		Scr		4503.00	140	7.55	76.70	5.79	15.90	1.20	6.20	0.47	0.80	0.06	319	363	83		
01007		TAB		Scr		1394.00	205	5.78	73.00	4.24	13.00	0.76	6.50	0.37	6.00	0.35	376	127		14.1	43
01008		CHB		Scr		70.70	229	4.67	54.00	2.56	32.00	1.52	8.30	0.39	3.40	0.16	461	499		15.6	45
01009	003	RAB		Scr		238.60	144	3.70	78.00	2.88	14.00	0.55	5.40	0.20	1.20	0.04	211	162	43	9.8	30
01009	003	RAB		0		336.90	123	3.92	68.00	2.67	21.00	0.83	8.70	0.34	1.50	0.06	374	188		10.9	31
01009	003	RAB		1			122	3.35	71.00	2.38	22.00	0.74	4.00	0.14	1.80	0.06	307	192		11.3	32
01009	003	RAB		2			109	4.05	74.00	2.99	19.00	0.77	4.80	0.20	1.20	0.05	328	220		11.3	33
01009	003	RAB		3			114	3.79	70.00	2.67	21.00	0.81	6.20	0.23	1.30	0.05	313	265		10.9	31
01009	003	RAB		7		249.30	69	3.38	75.00	2.54	17.00	0.60	5.60	0.19	0.70	0.02	244	161		10.4	30
01009	003	RAB		14		269.80	101	3.68	69.00	2.54	21.20	0.78	8.50	0.31	1.00	0.04	308	173		8.8	25
01009	003	RAB		21			122	4.82	78.00	3.76	13.20	0.64	7.70	0.37	0.60	0.03	218	195		7.4	20
01012	004	CEH		Scr		112.90	172	5.85	64.00	3.74	28.00	1.69	5.60	0.33	1.00	0.06	746	451	10	13.2	40
01012	004	CEH		1													642	475			
01012	004	CEH		2			150	4.82	67.70	3.26	26.40	1.28	4.60	0.22	1.10	0.05	552	380		12.2	36
01012	004	CEH		3			147	4.36	83.70	2.78	29.30	1.28	5.10	0.22	1.30	0.06	544	441		13.1	37
01012	004	CEH		7		190.00	159	4.95	58.60	2.90	32.70	1.61	5.90	0.29	2.50	0.12	642	506		12.6	35
01012	004	CEH		14		207.60	199	5.64	63.10	3.55	29.30	1.65	5.70	0.32	1.60	0.09				13.5	38
01013		KJF		Scr		49.10	228	8.53	65.00	5.62	26.00	2.23	5.30	0.46	2.30	0.20	1213	398		13.4	37
02014	002	L-S		Scr		12.70	222	5.65	53.00	3.01	34.00	1.92	7.40	0.42	3.90	0.22	721	439		13.6	40
02014	002	L-S		0		27.50	217	5.88	57.00	3.36	32.00	1.88	8.60	0.50	1.50	0.09	676	389		13.5	38
02014	002	L-S		1			226	5.74	55.00	3.19	35.00	2.04	7.00	0.40	1.40	0.08	632	405		13.6	38
02014	002	L-S		2			223	5.59	55.00	3.09	32.00	1.84	9.80	0.55	1.40	0.08	590	339		13.5	39
02014	002	L-S		3			219	4.89	54.00	2.66	34.00	1.68	7.50	0.37	2.70	0.13	529	358		13.2	37
01016	ineligible	G-F		Scr		4856.00	106	7.31	86.00	6.29	5.00	0.33	6.80	0.49	1.90	0.14	57.6	7		10.3	31
			normal range		low		150	3.80	40.50	1.96	15.40	0.80	2.60	0.12			404	220			
					high	7.00		10.70	75.00	7.23	48.50	3.00	10.00	0.92	6.80	0.57	1612	1128	30		

TABLE 13

Study No. MDXCTLA4-02
Selected Lab Values Summary

						Platelets	WBC	Neuts		Lymphs		Monos	
Screen no.	Subject no.	Initials	Amendment #	Day	Date	$\times 10^3$/ul	$\times 10^3$/ul	%	$\times 10^3$/ul	%	$\times 10^3$/ul	%	$\times 10^3$/ul
02001	001	SAH	0	Scr		216	6.28	56.60	3.52	35.60	2.23	5.90	0.37
02001	001	SAH	0	0		230	5.58	59.70	3.33	32.30	1.80	5.70	0.32
02001	001	SAH	0	1		202	5.12	61.80	3.16	30.20	1.55	5.00	0.26
			normal range		low	150	3.80	40.50	1.96	15.40	0.80	2.60	0.12
					high		10.70	75.00	7.23	48.50	3.00	10.10	0.92

						Eos		CD4/	CD8/	ESR	Hgb	Hcrit
Screen no.	Subject no.	Initials	Amendment #	Day	Date	%	$\times 10^3$/ul	ul	ul	mm/hr	g/dl	%
02001	001	SAH	0	Scr		1.80	0.11	1189	631		14.4	39
02001	001	SAH	0	0		1.80	0.10	1039	502		14.9	43
02001	001	SAH	0	1		2.30	0.12	957	407		13.4	37
			normal range		low			404	220			
					high	6.80	0.57	1612	1129	30		

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 41

<210> SEQ ID NO 1
<211> LENGTH: 3159
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:cloning vector pGP1k

<400> SEQUENCE: 1

aattagcggc cgctgtcgac aagcttcgaa ttcagtatcg atgtggggta cctactgtcc 60

cgggattgcg gatccgcgat gatatcgttg atcctcgagt gcggccgcag tatgcaaaaa 120

aaagcccgct cattaggcgg gctcttggca gaacatatcc atcgcgtccg ccatctccag 180

cagccgcacg cggcgcatct cgggcagcgt tgggtcctgg ccacgggtgc gcatgatcgt 240

gctcctgtcg ttgaggaccc ggctaggctg gcggggttgc cttactggtt agcagaatga 300

atcaccgata cgcgagcgaa cgtgaagcga ctgctgctgc aaaacgtctg cgacctgagc 360

aacaacatga atggtcttcg gtttccgtgt ttcgtaaagt ctggaaacgc ggaagtcagc 420

gccctgcacc attatgttcc ggatctgcat cgcaggatgc tgctggctac cctgtggaac 480

acctacatct gtattaacga agcgctggca ttgaccctga gtgatttttc tctggtcccg 540

ccgcatccat accgccagtt gtttaccctc acaacgttcc agtaaccggg catgttcatc 600

atcagtaacc cgtatcgtga gcatcctctc tcgtttcatc ggtatcatta cccccatgaa 660

cagaaattcc cccttacacg gaggcatcaa gtgaccaaac aggaaaaaac cgcccttaac 720

atggcccgct ttatcagaag ccagacatta acgcttctgg agaaactcaa cgagctggac 780

gcggatgaac aggcagacat ctgtgaatcg cttcacgacc acgctgatga gctttaccgc 840

agctgcctcg cgcgtttcgg tgatgacggt gaaaacctct gacacatgca gctcccggag 900

acggtcacag cttgtctgta agcggatgcc gggagcagac aagcccgtca gggcgcgtca 960

gcgggtgttg gcgggtgtcg gggcgcagcc atgacccagt cacgtagcga tagcggagtg 1020

tatactggct taactatgcg gcatcagagc agattgtact gagagtgcac catatgcggt 1080

-continued

gtgaaatacc gcacagatgc gtaaggagaa aataccgcat caggcgctct tccgcttcct 1140

cgctcactga ctcgctgcgc tcggtcgttc ggctgcggcg agcggtatca gctcactcaa 1200

aggcggtaat acggttatcc acagaatcag gggataacgc aggaaagaac atgtgagcaa 1260

aaggccagca aaaggccagg aaccgtaaaa aggccgcgtt gctggcgttt ttccataggc 1320

tccgcccccc tgacgagcat cacaaaaatc gacgctcaag tcagaggtgg cgaaacccga 1380

caggactata aagataccag gcgtttcccc ctggaagctc cctcgtgcgc tctcctgttc 1440

cgaccctgcc gcttaccgga tacctgtccg cctttctccc ttcgggaagc gtggcgcttt 1500

ctcatagctc acgctgtagg tatctcagtt cggtgtaggt cgttcgctcc aagctgggct 1560

gtgtgcacga acccccccgtt cagcccgacc gctgcgcctt atccggtaac tatcgtcttg 1620

agtccaaccc ggtaagacac gacttatcgc cactggcagc agccaggcgc gccttggcct 1680

aagaggccac tggtaacagg attagcagag cgaggtatgt aggcggtgct acagagttct 1740

tgaagtggtg gcctaactac ggctacacta gaaggacagt atttggtatc tgcgctctgc 1800

tgaagccagt taccttcgga aaaagagttg gtagctcttg atccggcaaa caaaccaccg 1860

ctggtagcgg tggtttttttt gtttgcaagc agcagattac gcgcagaaaa aaaggatctc 1920

aagaagatcc tttgatcttt tctacggggt ctgacgctca gtggaacgaa aactcacgtt 1980

aagggatttt ggtcatgaga ttatcaaaaa ggatcttcac ctagatcctt ttaaattaaa 2040

aatgaagttt taaatcaatc taaagtatat atgagtaaac ttggtctgac agttaccaat 2100

gcttaatcag tgaggcacct atctcagcga tctgtctatt tcgttcatcc atagttgcct 2160

gactccccgt cgtgtagata actacgatac gggagggctt accatctggc cccagtgctg 2220

caatgatacc gcgagaccca cgctcaccgg ctccagattt atcagcaata aaccagccag 2280

ccggaagggc cgagcgcaga agtggtcctg caactttatc cgcctccatc cagtctatta 2340

attgttgccg ggaagctaga gtaagtagtt cgccagttaa tagtttgcgc aacgttgttg 2400

ccattgctgc aggcatcgtg gtgtcacgct cgtcgtttgg tatggcttca ttcagctccg 2460

gttcccaacg atcaaggcga gttacatgat cccccatgtt gtgcaaaaaa gcggttagct 2520

ccttcggtcc tccgatcgtt gtcagaagta agttggccgc agtgttatca ctcatggtta 2580

tggcagcact gcataattct cttactgtca tgccatccgt aagatgcttt tctgtgactg 2640

gtgagtactc aaccaagtca ttctgagaat agtgtatgcg gcgaccgagt tgctcttgcc 2700

cggcgtcaac acgggataat accgcgccac atagcagaac tttaaaagtg ctcatcattg 2760

gaaaacgttc ttcggggcga aaactctcaa ggatcttacc gctgttgaga tccagttcga 2820

tgtaacccac tcgtgcaccc aactgatctt cagcatcttt tactttcacc agcgtttctg 2880

ggtgagcaaa aacaggaagg caaaatgccg caaaaaaggg aataagggcg acacggaaat 2940

gttgaatact catactcttc ctttttcaat attattgaag catttatcag ggttattgtc 3000

tcatgagcgg atacatattt gaatgtattt agaaaaataa acaaataggg gttccgcgca 3060

catttccccg aaaagtgcca cctgacgtct aagaaaccat tattatcatg acattaacct 3120

ataaaaatag gcgtatcacg aggccctttc gtcttcaag 3159

<210> SEQ ID NO 2
<211> LENGTH: 349
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: preliminary sequence for heavy chain fragment 10D1.3

-continued

<400> SEQUENCE: 2

tgggggaggc gtggtccagc ctgggaggtc cctgagactc tcctgtgcag cctctggatt 60

caccttcagt agctatacta tgcactgggt ccgccaggct ccaggcaagg ggctggagtg 120

ggtgacattt atatcatatg atggaaacaa taaatactac gcagactccg tgaagggccg 180

attcaccatc tccagagaca attccaagaa cacgctgtat ctgcaaatga acagcctgag 240

agctgaggac acggctatat attactgtgc gaggaccggc tggctggggc cctttgacta 300

ctggggccag ggaaccctgg tcaccgtctc ctcagcctcc accaagggc 349

<210> SEQ ID NO 3
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: preliminary sequence for light chain fragment 10D1.3

<400> SEQUENCE: 3

ctccaggcac cctgtctttg tctccagggg aaagagccac cctctcctgc agggccagtc 60

agagtgttgg cagcagctac ttagcctggt accagcagaa acctggccag gctcccaggc 120

tcctcatcta tggtgcattc agcagggcca ctggcatccc agacaggttc agtggcagtg 180

ggtctgggac agacttcact ctcaccatca gcagactgga gcctgaagat tttgcagtgt 240

attactgtca gcagtatggt agctcaccgt ggacgttcgg ccaagggacc aaggtggaaa 300

tcaaacgaac tgtggctgca c 321

<210> SEQ ID NO 4
<211> LENGTH: 287
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Vk A-27 germline sequence

<400> SEQUENCE: 4

gaaattgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60

ctctcctgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa 120

cctggccagg ctcccaggct cctcatctat ggtgcatcca gcagggccac tggcatccca 180

gacaggttca gtggcagtgg gtctgggaca gacttcactc tcaccatcag cagactggag 240

cctgaagatt ttgcagtgta ttactgtcag cagtatggta gctcacc 287

<210> SEQ ID NO 5
<211> LENGTH: 95
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain variable region predicted sequence for Vk A-27 germline

<400> SEQUENCE: 5

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

-continued

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser
85 90 95

<210> SEQ ID NO 6
<211> LENGTH: 325
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain variable region (Vk), 10D1 from Vk A-27

<400> SEQUENCE: 6

gaaattgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60

ctctcctgca gggccagtca gagtgttggc agcagctact tagcctggta ccagcagaaa 120

cctggccagg ctcccaggct cctcatctat ggtgcattca gcagggccac tggcatccca 180

gacaggttca gtggcagtgg gtctgggaca gacttcactc tcaccatcag cagactggag 240

cctgaagatt ttgcagtgta ttactgtcag cagtatggta gctcaccgtg gacgttcggc 300

caagggacca aggtggaaat caaac 325

<210> SEQ ID NO 7
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain variable region predicted sequence for 10D1 from Vk A-27

<400> SEQUENCE: 7

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Gly Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Phe Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
85 90 95

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> SEQ ID NO 8
<211> LENGTH: 325
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain variable region (Vk) 4B6 from Vk A-27

<400> SEQUENCE: 8

gaaattgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60

ctctcctgca gggccagtca gagtgttagc agcagcttct tagcctggta ccagcagaaa 120

cctggccagg ctcccaggct cctcatctat ggtgcatcca gcagggccac tggcatccca 180

-continued

gacaggttca gtggcagtgg gtctgggaca gacttcactc tcaccatcag cagactggag 240

cctgaagatt ttgcagtgta ttactgtcag cagtatggta gctcaccgtg gacgttcggc 300

caagggacca aggtggaaat caaac 325

<210> SEQ ID NO 9
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain variable region predicted sequence
for 4B6 from Vk A-27

<400> SEQUENCE: 9

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Phe Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
85 90 95

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> SEQ ID NO 10
<211> LENGTH: 287
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Vk L-15 germline sequence

<400> SEQUENCE: 10

gacatccaga tgacccagtc tccatcctca ctgtctgcat ctgtaggaga cagagtcacc 60

atcacttgtc gggcgagtca gggtattagc agctggttag cctggtatca gcagaaacca 120

gagaaagccc ctaagtccct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180

aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240

gaagattttg caacttatta ctgccaacag tataatagtt accctcc 287

<210> SEQ ID NO 11
<211> LENGTH: 94
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain variable region predicted sequence
for Vk L-15 germline

<400> SEQUENCE: 11

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly

-continued

```
     50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr
                 85                  90
```

<210> SEQ ID NO 12
<211> LENGTH: 322
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain variable region Vk 1E2 from Vk L-15

<400> SEQUENCE: 12

```
gacatccaga tgacccagtc tccatcctca ctgtctgcat ctgtaggaga cagagtcacc     60

atcacttgtc gggcgagtca gggtattagc agctggttag cctggtatca gcagaaacca    120

gagaaagccc ctaagtccct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca    180

aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct    240

gaagattttg caacttatta ctgccaacag tataatagtt accctccgac gttcggccaa    300

gggaccaagg tggaaatcaa ac                                             322
```

<210> SEQ ID NO 13
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain variable region predicted sequence
for 1E2 from Vk L-15

<400> SEQUENCE: 13

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
  1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
             20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
         35                  40                  45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
     50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Pro
                 85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> SEQ ID NO 14
<211> LENGTH: 294
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: VH 3-30.3 germline sequence

<400> SEQUENCE: 14

```
caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc     60

tcctgtgcag cctctggatt caccttcagt agctatgcta tgcactgggt ccgccaggct    120

ccaggcaagg ggctggagtg ggtggcagtt atatcatatg atggaagcaa taaatactac    180

gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat    240
```

-continued

ctgcaaatga acagcctgag agctgaggac acggctgtgt attactgtgc gaga 294

<210> SEQ ID NO 15
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain variable region predicted sequence for VH 3-30.3 germline

<400> SEQUENCE: 15

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> SEQ ID NO 16
<211> LENGTH: 355
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain variable region VH 10D1 from VH 3-30.3

<400> SEQUENCE: 16

caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60

tcctgtgcag cctctggatt caccttcagt agctatacta tgcactgggt ccgccaggct 120

ccaggcaagg ggctggagtg ggtgacattt atatcatatg atggaaacaa taaatactac 180

gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agctgaggac acggctatat attactgtgc gaggaccggc 300

tggctggggc cctttgacta ctggggccag ggaaccctgg tcaccgtctc ctcag 355

<210> SEQ ID NO 17
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain variable region predicted sequence for 10D1 from VH 3-30.3

<400> SEQUENCE: 17

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Thr Phe Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

-continued

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Thr Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> SEQ ID NO 18
<211> LENGTH: 355
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain variable region VH 4B6 from VH 3-30.3

<400> SEQUENCE: 18

caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60

tcctgtgcag cctctggatt caccttcagt agctatacta tgcactgggt ccgccaggct 120

ccaggcaagg ggctggagtg ggtgacattt atatcatatg atggaagcaa taaacactac 180

gcagactccg tgaagggccg attcaccgtc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agctgaggac acggctatat attactgtgc gaggaccggc 300

tggctggggc cctttgacta ctggggccag ggaaccctgg tcaccgtctc ctcag 355

<210> SEQ ID NO 19
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain variable region predicted sequence for 4B6 from VH 3-30.3

<400> SEQUENCE: 19

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Thr Phe Ile Ser Tyr Asp Gly Ser Asn Lys His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Thr Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> SEQ ID NO 20
<211> LENGTH: 296
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: VH 3-33 germline sequence

-continued

<400> SEQUENCE: 20

caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60

tcctgtgcag cgtctggatt caccttcagt agctatggca tgcactgggt ccgccaggct 120

ccaggcaagg ggctggagtg ggtggcagtt atatggtatg atggaagtaa taaatactat 180

gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagaga 296

<210> SEQ ID NO 21
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain variable region predicted sequence for VH 3-33 germline

<400> SEQUENCE: 21

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> SEQ ID NO 22
<211> LENGTH: 358
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain variable region VH 1E2 from VH 3-33

<400> SEQUENCE: 22

caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60

tcctgtgcag cgtctggatt caccttcagt agctatggca tgcactgggt ccgccaggct 120

ccaggcaagg ggctggagtg ggtggcagtt atatggtatg atggaagtaa taaatactat 180

gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt tttactgtgc gagagctccc 300

aattatattg gtgcttttga tgtctggggc caagggacaa tggtcaccgt ctcttcag 358

<210> SEQ ID NO 23
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain variable region predicted sequence for 1E2 from VH 3-33

<400> SEQUENCE: 23

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

-continued

```
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Phe Tyr Cys
                85                  90                  95

Ala Arg Ala Pro Asn Tyr Ile Gly Ala Phe Asp Val Trp Gly Gln Gly
            100                 105                 110

Thr Met Val Thr Val Ser Ser
        115


<210> SEQ ID NO 24
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain CDR1 (HuMab 10D1)

<400> SEQUENCE: 24

Arg Ala Ser Gln Ser Val Gly Ser Ser Tyr Leu Ala
  1               5                   10


<210> SEQ ID NO 25
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain CDR1 (HuMab 4B6)

<400> SEQUENCE: 25

Arg Ala Ser Gln Ser Val Ser Ser Ser Phe Leu Ala
  1               5                   10


<210> SEQ ID NO 26
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain CDR1 (HuMab 1E2)

<400> SEQUENCE: 26

Arg Ala Ser Gln Gly Ile Ser Ser Trp Leu Ala
  1               5                   10


<210> SEQ ID NO 27
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain CDR1 (HuMab 10D1, 4B6)

<400> SEQUENCE: 27

Ser Tyr Thr Met His
  1               5


<210> SEQ ID NO 28
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: heavy chain CDR1 (HuMab 1E2)

<400> SEQUENCE: 28

Ser Tyr Gly Met His
1 5

<210> SEQ ID NO 29
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain CDR2 (HuMab 10D1)

<400> SEQUENCE: 29

Gly Ala Phe Ser Arg Ala Thr
1 5

<210> SEQ ID NO 30
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain CDR2 (HuMab 4B6)

<400> SEQUENCE: 30

Gly Ala Ser Ser Arg Ala Thr
1 5

<210> SEQ ID NO 31
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain CDR2 (HuMab 1E2)

<400> SEQUENCE: 31

Ala Ala Ser Ser Leu Gln Ser
1 5

<210> SEQ ID NO 32
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain CDR2 (HuMab 10D1)

<400> SEQUENCE: 32

Phe Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> SEQ ID NO 33
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain CDR2 (HuMab 4B6)

<400> SEQUENCE: 33

Phe Ile Ser Tyr Asp Gly Ser Asn Lys His Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> SEQ ID NO 34
<211> LENGTH: 17

-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain CDR2 (HuMab 1E2)

<400> SEQUENCE: 34

Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> SEQ ID NO 35
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain CDR3 (HuMab 10D1, 4B6)

<400> SEQUENCE: 35

Gln Gln Tyr Gly Ser Ser Pro Trp Thr
1 5

<210> SEQ ID NO 36
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: light chain CDR3 (HuMab 1E2)

<400> SEQUENCE: 36

Gln Gln Tyr Asn Ser Tyr Pro Pro Thr
1 5

<210> SEQ ID NO 37
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain CDR3 (HuMab 10D1, 4B6)

<400> SEQUENCE: 37

Thr Gly Trp Leu Gly Pro Phe Asp Tyr
1 5

<210> SEQ ID NO 38
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: heavy chain CDR3 (MuMab 1E2)

<400> SEQUENCE: 38

Ala Pro Asn Tyr Ile Gly Ala Phe Asp Val
1 5 10

<210> SEQ ID NO 39
<211> LENGTH: 506
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:kappa light chain plasmid pCK7-96 (partial)

<400> SEQUENCE: 39

aggagaatga ataaataaag tgaatctttg cacctgtggt ttctctcttt cctcaattta 60

ataattatta tctgttgttt accaactact caatttctct tataagggac taaatatgta 120

-continued

gtcatcctaa ggcgcataac catttataaa aatcatcctt cattctattt taccctatca 180

tcctctgcaa gacagtcctc cctcaaaccc acaagccttc tgtcctcaca gtcccctggg 240

ccatggatcc tcacatccca atccgcggcc gcaattcgta atcatggtca tagctgtttc 300

ctgtgtgaaa ttgttatccg ctcacaattc cacacaacat acgagccgga agcataaagt 360

gtaaagcctg gggtgcctaa tgagtgagct aactcacatt aattgcgttg cgctcactgc 420

ccgctttcca gtcgggaaac ctgtcgtgcc agctgcatta atgaatcggc caacgcgcgg 480

ggagaggcgg tttgcgtatt gggcgc 506

<210> SEQ ID NO 40
<211> LENGTH: 4723
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:gamma1 heavy chain plasmid pCG-96

<400> SEQUENCE: 40

gaactcgagc agctgaagct ttctggggca ggccaggcct gaccttggct ttggggcagg 60

gaggggcta aggtgaggca ggtggcgcca gccaggtgca cacccaatgc ccatgagccc 120

agacactgga cgctgaacct cgcggacagt taagaaccca ggggcctctg cgccctgggc 180

ccagctctgt cccacaccgc ggtcacatgg caccacctct cttgcagcct ccaccaaggg 240

cccatcggtc ttccccctgg caccctcctc caagagcacc tctgggggca cagcggccct 300

gggctgcctg gtcaaggact acttccccga accggtgacg gtgtcgtgga actcaggcgc 360

cctgaccagc ggcgtgcaca ccttcccggc tgtcctacag tcctcaggac tctactccct 420

cagcagcgtg gtgaccgtgc cctccagcag cttgggcacc cagacctaca tctgcaacgt 480

gaatcacaag cccagcaaca ccaaggtgga caagaaagtt ggtgagaggc cagcacaggg 540

agggagggtg tctgctggaa gccaggctca gcgctcctgc ctggacgcat cccggctatg 600

cagccccagt ccagggcagc aaggcaggcc ccgtctgcct cttcacccgg aggcctctgc 660

ccgccccact catgctcagg gagagggtct tctggctttt tccccaggct ctgggcaggc 720

acaggctagg tgcccctaac ccaggccctg cacacaaagg ggcaggtgct gggctcagac 780

ctgccaagag ccatatccgg gaggaccctg cccctgacct aagcccaccc caaaggccaa 840

actctccact ccctcagctc ggacaccttc tctcctccca gattccagta actcccaatc 900

ttctctctgc agagcccaaa tcttgtgaca aaactcacac atgcccaccg tgcccaggta 960

agccagccca ggcctcgccc tccagctcaa ggcgggacag gtgccctaga gtagcctgca 1020

tccagggaca ggccccagcc gggtgctgac acgtccacct ccatctcttc ctcagcacct 1080

gaactcctgg ggggaccgtc agtcttcctc ttcccccaa aacccaagga caccctcatg 1140

atctcccgga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag 1200

gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg 1260

gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac 1320

tggctgaatg gcaaggagta caagtgcaag gtctccaaca aagccctccc agcccccatc 1380

gagaaaacca tctccaaagc caaaggtggg acccgtgggg tgcgagggcc acatggacag 1440

aggccggctc ggcccaccct ctgccctgag agtgaccgct gtaccaacct ctgtccctac 1500

agggcagccc cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa 1560

gaaccaggtc agcctgacct gcctggtcaa aggcttctat cccagcgaca tcgccgtgga 1620

-continued

gtgggagagc aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc 1680

cgacggctcc ttcttcctct acagcaagct caccgtggac aagagcaggt ggcagcaggg 1740

gaacgtcttc tcatgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag 1800

cctctccctg tctccgggta aatgagtgcg acggccggca agcccccgct ccccgggctc 1860

tcgcggtcgc acgaggatgc ttggcacgta ccccctgtac atacttcccg ggcgcccagc 1920

atggaaataa agcacccagc gctgccctgg gcccctgcga gactgtgatg gttctttcca 1980

cgggtcaggc cgagtctgag gcctgagtgg catgagggag gcagagcggg tcccactgtc 2040

cccacactgg cccaggctgt gcaggtgtgc ctgggccccc tagggtgggg ctcagccagg 2100

ggctgccctc ggcagggtgg gggatttgcc agcgtggccc tccctccagc agcacctgcc 2160

ctgggctggg ccacgggaag ccctaggagc ccctggggac agacacacag cccctgcctc 2220

tgtaggagac tgtcctgttc tgtgagcgcc cctgtcctcc cgacctccat gcccactcgg 2280

gggcatgcct gcaggtcgac tctagaggat ccccgggtac cgagctcgaa ttcatcgatg 2340

atatcagatc tgccggtctc cctatagtga gtcgtattaa tttcgataag ccaggttaac 2400

ctgcattaat gaatcggcca acgcgcgggg agaggcggtt tgcgtattgg gcgctcttcc 2460

gcttcctcgc tcactgactc gctgcgctcg gtcgttcggc tgcggcgagc ggtatcagct 2520

cactcaaagg cggtaatacg gttatccaca gaatcagggg ataacgcagg aaagaacatg 2580

tgagcaaaag gccagcaaaa ggccaggaac cgtaaaaagg ccgcgttgct ggcgtttttc 2640

cataggctcc gccccctga cgagcatcac aaaaatcgac gctcaagtca gaggtggcga 2700

aacccgacag gactataaag ataccaggcg tttcccctg gaagctccct cgtgcgctct 2760

cctgttccga ccctgccgct taccggatac ctgtccgcct ttctcccttc gggaagcgtg 2820

gcgctttctc aatgctcacg ctgtaggtat ctcagttcgg tgtaggtcgt tcgctccaag 2880

ctgggctgtg tgcacgaacc ccccgttcag cccgaccgct gcgccttatc cggtaactat 2940

cgtcttgagt ccaacccggt aagacacgac ttatcgccac tggcagcagc cactggtaac 3000

aggattagca gagcgaggta tgtaggcggt gctacagagt tcttgaagtg gtggcctaac 3060

tacggctaca ctagaaggac agtatttggt atctgcgctc tgctgaagcc agttaccttc 3120

ggaaaaagag ttggtagctc ttgatccggc aaacaaacca ccgctggtag cggtggtttt 3180

tttgtttgca agcagcagat tacgcgcaga aaaaaaggat ctcaagaaga tcctttgatc 3240

ttttctacgg ggtctgacgc tcagtggaac gaaaactcac gttaagggat tttggtcatg 3300

agattatcaa aaaggatctt cacctagatc cttttaaatt aaaaatgaag ttttaaatca 3360

atctaaagta tatatgagta aacttggtct gacagttacc aatgcttaat cagtgaggca 3420

cctatctcag cgatctgtct atttcgttca tccatagttg cctgactccc cgtcgtgtag 3480

ataactacga tacgggaggg cttaccatct ggccccagtg ctgcaatgat accgcgagac 3540

ccacgctcac cggctccaga tttatcagca ataaaccagc cagccggaag ggccgagcgc 3600

agaagtggtc ctgcaacttt atccgcctcc atccagtcta ttaattgttg ccgggaagct 3660

agagtaagta gttcgccagt taatagtttg cgcaacgttg ttgccattgc tacaggcatc 3720

gtggtgtcac gctcgtcgtt tggtatggct tcattcagct ccggttccca acgatcaagg 3780

cgagttacat gatcccccat gttgtgcaaa aaagcggtta gctccttcgg tcctccgatc 3840

gttgtcagaa gtaagttggc cgcagtgtta tcactcatgg ttatggcagc actgcataat 3900

tctcttactg tcatgccatc cgtaagatgc ttttctgtga ctggtgagta ctcaaccaag 3960

tcattctgag aatagtgtat gcggcgaccg agttgctctt gcccggcgtc aatacgggat 4020

-continued

aataccgcgc cacatagcag aactttaaaa gtgctcatca ttggaaaacg ttcttcgggg 4080

cgaaaactct caaggatctt accgctgttg agatccagtt cgatgtaacc cactcgtgca 4140

cccaactgat cttcagcatc ttttactttc accagcgttt ctgggtgagc aaaaacagga 4200

aggcaaaatg ccgcaaaaaa gggaataagg gcgacacgga aatgttgaat actcatactc 4260

ttccttttc aatattattg aagcatttat cagggttatt gtctcatgag cggatacata 4320

tttgaatgta tttagaaaaa taaacaaata ggggttccgc gcacatttcc ccgaaaagtg 4380

ccacctgacg tctaagaaac cattattatc atgacattaa cctataaaaa taggcgtatc 4440

acgaggccct ttcgtctcgc gcgtttcggt gatgacggtg aaaacctctg acacatgcag 4500

ctcccggaga cggtcacagc ttgtctgtaa gcggatgccg ggagcagaca agcccgtcag 4560

ggcgcgtcag cgggtgttgg cgggtgtcgg ggctggctta actatgcggc atcagagcag 4620

attgtactga gagtgcacca tatggacata ttgtcgttag aacgcggcta caattaatac 4680

ataaccttat gtatcataca catacgattt aggtgacact ata 4723

<210> SEQ ID NO 41
<211> LENGTH: 4694
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:gamma4 heavy chain plasmid pG4HE

<400> SEQUENCE: 41

gaactcgagc agctgaagct ttctggggca ggccgggcct gactttggct ggggcaggg 60

aggggctaa ggtgacgcag gtggcgccag ccaggtgcac acccaatgcc catgagccca 120

gacactggac cctgcatgga ccatcgcgga tagacaagaa ccgaggggcc tctgcgccct 180

gggcccagct ctgtcccaca ccgcggtcac atggcaccac ctctcttgca gcttccacca 240

agggcccatc cgtcttcccc ctggcgccct gctccaggag cacctccgag agcacagccg 300

ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg tggaactcag 360

gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca ggactctact 420

ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacgaagacc tacacctgca 480

acgtagatca caagcccagc aacaccaagg tggacaagag agttggtgag aggccagcac 540

agggagggag ggtgtctgct ggaagccagg ctcagccctc ctgcctggac gcaccccggc 600

tgtgcagccc cagcccaggg cagcaaggca tgccccatct gtctcctcac ccggaggcct 660

ctgaccaccc cactcatgct cagggagagg gtcttctgga tttttccacc aggctccggg 720

cagccacagg ctggatgccc ctaccccagg ccctgcgcat acaggggcag gtgctgcgct 780

cagacctgcc aagagccata tccgggagga ccctgcccct gacctaagcc caccccaaag 840

gccaaactct ccactccctc agctcagaca ccttctctcc tcccagatct gagtaactcc 900

caatcttctc tctgcagagt ccaaatatgg tcccccatgc ccatcatgcc caggtaagcc 960

aacccaggcc tcgccctcca gctcaaggcg ggacaggtgc cctagagtag cctgcatcca 1020

gggacaggcc ccagccgggt gctgacgcat ccacctccat ctcttcctca gcacctgagt 1080

tcctgggggg accatcagtc ttcctgttcc ccccaaaacc caaggacact ctcatgatct 1140

cccggacccc tgaggtcacg tgcgtggtgg tggacgtgag ccaggaagac cccgaggtcc 1200

agttcaactg gtacgtggat ggcgtggagg tgcataatgc caagacaaag ccgcgggagg 1260

agcagttcaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac caggactggc 1320

-continued

tgaacggcaa ggagtacaag tgcaaggtct ccaacaaagg cctcccgtcc tccatcgaga 1380

aaaccatctc caaagccaaa ggtgggaccc acggggtgcg agggccacat ggacagaggt 1440

cagctcggcc caccctctgc cctgggagtg accgctgtgc caacctctgt ccctacaggg 1500

cagccccgag agccacaggt gtacaccctg cccccatccc aggaggagat gaccaagaac 1560

caggtcagcc tgacctgcct ggtcaaaggc ttctacccca gcgacatcgc cgtggagtgg 1620

gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac 1680

ggctccttct tcctctacag caggctaacc gtggacaaga gcaggtggca ggaggggaat 1740

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacaca gaagagcctc 1800

tccctgtctc tgggtaaatg agtgccaggg ccggcaagcc cccgctcccc gggctctcgg 1860

ggtcgcgcga ggatgcttgg cacgtacccc gtctacatac ttcccaggca cccagcatgg 1920

aaataaagca cccaccactg ccctgggccc ctgtgagact gtgatggttc tttccacggg 1980

tcaggccgag tctgaggcct gagtgacatg agggaggcag agcgggtccc actgtcccca 2040

cactggccca ggctgtgcag gtgtgcctgg gccacctagg gtggggctca gccaggggct 2100

gccctcggca gggtgggggа tttgccagcg tggccctccc tccagcagca gctgccctgg 2160

gctgggccac gggaagccct aggagcccct ggggacagac acacagcccc tgcctctgta 2220

ggagactgtc ctgtcctgtg agcgccctgt cctccgaccc cccatgccca ctcgggggga 2280

tccccgggta ccgagctcga attcatcgat gatatcagat ctgccggtct ccctatagtg 2340

agtcgtatta atttcgataa gccaggttaa cctgcattaa tgaatcggcc aacgcgcggg 2400

gagaggcggt ttgcgtattg ggcgctcttc cgcttcctcg ctcactgact cgctgcgctc 2460

ggtcgttcgg ctgcggcgag cggtatcagc tcactcaaag gcggtaatac ggttatccac 2520

agaatcaggg gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa 2580

ccgtaaaaag gccgcgttgc tggcgttttt ccataggctc cgccccccctg acgagcatca 2640

caaaaatcga cgctcaagtc agaggtggcg aaacccgaca ggactataaa gataccaggc 2700

gtttcccccct ggaagctccc tcgtgcgctc tcctgttccg accctgccgc ttaccggata 2760

cctgtccgcc tttctccctt cgggaagcgt ggcgctttct caatgctcac gctgtaggta 2820

tctcagttcg gtgtaggtcg ttcgctccaa gctgggctgt gtgcacgaac cccccgttca 2880

gcccgaccgc tgcgccttat ccggtaacta tcgtcttgag tccaacccgg taagacacga 2940

cttatcgcca ctggcagcag ccactggtaa caggattagc agagcgaggt atgtaggcgg 3000

tgctacagag ttcttgaagt ggtggcctaa ctacggctac actagaagga cagtatttgg 3060

tatctgcgct ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg 3120

caaacaaacc accgctggta gcggtggttt ttttgtttgc aagcagcaga ttacgcgcag 3180

aaaaaaagga tctcaagaag atcctttgat cttttctacg gggtctgacg ctcagtggaa 3240

cgaaaactca cgttaaggga ttttggtcat gagattatca aaaaggatct tcacctagat 3300

ccttttaaat taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc 3360

tgacagttac caatgcttaa tcagtgaggc acctatctca gcgatctgtc tatttcgttc 3420

atccatagtt gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc 3480

tggccccagt gctgcaatga taccgcgaga cccacgctca ccggctccag atttatcagc 3540

aataaaccag ccagccggaa gggccgagcg cagaagtggt cctgcaactt tatccgcctc 3600

catccagtct attaattgtt gccgggaagc tagagtaagt agttcgccag ttaatagttt 3660

-continued

```
gcgcaacgtt gttgccattg ctacaggcat cgtggtgtca cgctcgtcgt ttggtatggc   3720

ttcattcagc tccggttccc aacgatcaag gcgagttaca tgatccccca tgttgtgcaa   3780

aaaagcggtt agctccttcg gtcctccgat cgttgtcaga agtaagttgg ccgcagtgtt   3840

atcactcatg gttatggcag cactgcataa ttctcttact gtcatgccat ccgtaagatg   3900

cttttctgtg actggtgagt actcaaccaa gtcattctga gaatagtgta tgcggcgacc   3960

gagttgctct tgcccggcgt caatacggga taataccgcg ccacatagca gaactttaaa   4020

agtgctcatc attggaaaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt   4080

gagatccagt tcgatgtaac ccactcgtgc acccaactga tcttcagcat cttttacttt   4140

caccagcgtt tctgggtgag caaaaacagg aaggcaaaat gccgcaaaaa agggaataag   4200

ggcgacacgg aaatgttgaa tactcatact cttccttttt caatattatt gaagcattta   4260

tcagggttat tgtctcatga gcggatacat atttgaatgt atttagaaaa ataaacaaat   4320

aggggttccg cgcacatttc cccgaaaagt gccacctgac gtctaagaaa ccattattat   4380

catgacatta acctataaaa ataggcgtat cacgaggccc tttcgtctcg cgcgtttcgg   4440

tgatgacggt gaaaacctct gacacatgca gctcccggag acggtcacag cttgtctgta   4500

agcggatgcc gggagcagac aagcccgtca gggcgcgtca gcgggtgttg gcgggtgtcg   4560

gggctggctt aactatgcgg catcagagca gattgtactg agagtgcacc atatggacat   4620

attgtcgtta gaacgcggct acaattaata cataaccttа tgtatcatac acatacgatt   4680

taggtgacac tata                                                     4694
```

What is claimed is:

1. An antibody comprising:

(a) a heavy chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:19; and

(b) a light chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:9,

wherein the antibody is capable of binding human CTLA-4.

2. An antibody comprising:

(a) a heavy chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:17; and

(b) a light chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:7,

wherein the antibody is capable of binding human CTLA4.

3. An antibody comprising:

(a) a heavy chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:23; and

(b) a light chain variable amino acid sequence having the amino acid sequence set forth in SEQ ID NO:13,

wherein the antibody is capable of binding human CTLA4.

4. An antibody capable of binding human CTLA4, which antibody comprises:

(a) a heavy chain variable region having CDR sequences set forth in SEQ ID NOS:27, 32 and 37; and

(b) a light chain variable region having CDR sequences set forth in SEQ ID NOS:24, 29 and 35.

5. An antibody capable of binding human CTLA4, which antibody comprises:

(a) a heavy chain variable region having CDR sequences set forth in SEQ ID NOS:27, 33 and 37; and

(b) a light chain variable region having CDR sequences set forth in SEQ ID NOS:25, 30 and 35.

6. An antibody capable of binding human CTLA4, which antibody comprises:

(a) a heavy chain variable region having CDR sequences set forth in SEQ ID NOS:28, 34 and 38; and

(b) a light chain variable region having CDR sequences set forth in SEQ ID NOS:26, 31 and 36.

7. An antibody capable of binding human CTLA4, which antibody comprises:

(a) a heavy chain variable region of a human V_H 3-30.3 gene; and

(b) a light chain variable region of a human V_K A-27 gene.

8. An antibody according to claim 7, wherein the antibody is capable of binding human CTLA4 with a binding affinity of about 10^8 M^{-1} or greater.

9. An antibody according to claim 7, wherein the antibody is capable of binding human CTLA4 with a binding affinity of about 10^9 M^{-1} or greater.

10. An antibody according to claim 7, wherein the antibody inhibits binding of the human CTLA4 to B7-1 or to B7-2.

11. An antibody capable of binding human CTLA4, which antibody comprises:

(a) a heavy chain variable region of a human V_H 3-33 gene; and

(b) a light chain variable region of a human V_K L-15 gene.

12. An antibody according to claim 11, wherein the antibody is capable of binding human CTLA4 with a binding affinity of about 10^8 M^{-1} or greater.

13. An antibody according to claim 11, wherein the antibody is capable of binding human CTLA4 with a binding affinity of about 10^9 $M[-1]^{-1}$ or greater.

14. An antibody according to claim 11, wherein the antibody inhibits binding of the human CTLA4 to B7-1 or to B7-2.

* * * * *

AT Serafini
01464301051005

(2)

UNITED STATL DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

5034052

101726830A

AUGUST 06, 2001

PTAS

TOWNSEND AND TOWNSEND AND CREW LLP
ANDREW T. SERAFINI, PH.D.
TWO EMBARCADERO CENTER, 8TH FLOOR
SAN FRANCISCO, CA 94111-3834

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 05/18/2001 REEL/FRAME: 011817/0279
NUMBER OF PAGES: 2

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
KORMAN, ALAN J. DOC DATE: 05/15/2001

ASSIGNOR:
HALK, EDWARD L. DOC DATE: 05/15/2001

ASSIGNOR:
LONBERG, NILS DOC DATE: 05/15/2001

ASSIGNOR:
DEO, YASHWANT M. DOC DATE: 05/15/2001

ASSIGNOR:
KELER, TIBOR P. DOC DATE: 05/15/2001

ASSIGNEE:
MEDAREX, INC.
67 BEAVER AVENUE
ANNANDALE, NEW JERSEY 08801

SERIAL NUMBER: 09644668
PATENT NUMBER:

FILING DATE: 08/24/2000
ISSUE DATE:

LAZENA MARTIN, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

05-23-2001

101726830

PATENTS ONLY

Attorney Docket No. 014643010510

Form PTO-1595
(Rev. 03-01)
OMB No. 0651-0027 (exp. 5/31/2002)

RECORDATION FORM COVER SHEET

U.S. Department of Commerce
U.S. Patent and Trademark Office

To the Honorable Commissioner of Patents and Trademarks. Please record the attached original documents or copy thereof

1. Name of conveying party(ies):

Alan J. Korman, Edward L. Halk, Nils Lonberg, Yashwant M. Deo, and Tibor P. Keler

Additional name(s) of conveying parties attached? ☐ Yes ☒ No.

3. Nature of conveyance:

☒ Assignment ☐ Merger

☐ Security Agreement ☐ Change of Name

☐ Other: _____

Execution Date: May 15, 2001

2. Name and address of receiving party(ies)

Name: Medarex, Inc.

Internal Address: 67 Beaver Avenue

Annandale, NJ 08801

MAY 18 2001

Street Address: 67 Beaver Avenue

City: Annandale State: NJ ZIP: 08801

Additional names and addresses attached? ☐ Yes ☒ No

4. Application number(s) or patent number(s).

If this document is being filed together with a new application, the execution date of the application is: _____

A. Patent Application No(s): 09/644,668

B. Patent No(s):

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Andrew T. Serafini, Ph.D.
TOWNSEND AND TOWNSEND AND CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(415) 576-0200

6. Total number of applications and patents involved 1

7. Total fee (37 CFR 3.41): $40.00

☐ Enclosed

☒ Authorized to be charged to deposit account

8. Deposit account number: 20-1430

(Attach duplicate copy of this page if paying by deposit account)

DO NOT USE THIS SPACE

9. Statement and signature.

To the best of my knowledge and belief, the foregoing is true and correct and any attached copy is a true of copy of the original document.

Andrew T. Serafini, Ph.D.
Name of Person Signing
Atty. Reg. No. 41,303

Signature

May 16, 2001
Date

Total number of pages including cover sheet, attachments and document 3

Mail documents to be recorded with required cover to:
Commissioner of Patents and Trademarks, Box Assignments
Washington, D.C. 20231

PA 3145897 v1

Merck Ex. 1135, Pg. 105

Attorney Docket No.: 14643-010510US

ASSIGNMENT OF PATENT APPLICATION

JOINT

WHEREAS, Alan J. Korman, of 301 El Cerrito Avenue, Piedmont, CA 94611; Edward L. Halk, of 1004 Edmonds Court, Sunnyvale, CA 94087; Nils Lonberg, of 780 W. California Way, Woodside, CA 94062; Yashwant M. Deo, of 1545 Route 22 East, Annandale, NJ 08801; and Tibor P. Keler, of 30 Park Road, Ottsville, PA 18942 thereinafter referred to as "Assignors," are the inventors of the invention described and set forth in the below-identified application for United States Letters Patent:

Title of Invention:	HUMAN CTLA-4 ANTIBODIES AND THEIR USES
Filing Date:	August 24, 2000
Application No.:	09/644,668; and

WHEREAS, Medarex, Inc., located at 67 Beaver Avenue, Annandale, NJ, 08801, hereinafter referred to as "ASSIGNEE," is desirous of acquiring an interest in the invention and application and in any U.S. Letters Patent and Registrations which may be granted on the same;

For good and valuable consideration, receipt of which is hereby acknowledged by Assignors, Assignors have assigned, and by these presents do assign to Assignee all right, title and interest in and to the invention and application and to all foreign counterparts (including patent, utility model and industrial designs), and in and to any Letters Patent and Registrations which may hereafter be granted on the same in the United States and all countries throughout the world, and to claim the priority from the application as provided by the Paris Convention. The right, title and interest is to be held and enjoyed by Assignee and Assignee's successors and assigns as fully and exclusively as it would have been held and enjoyed by Assignors had this Assignment not been made, for the full term of any Letters Patent and Registrations which may be granted thereon, or of any division, renewal, continuation in whole or in part, substitution, conversion, reissue, prolongation or extension thereof.

Assignors further agree that they will, without charge to Assignee, but at Assignee's expense, (a) cooperate with Assignee in the prosecution of U.S. Patent applications and foreign counterparts on the invention and any improvements, (b) execute, verify, acknowledge and deliver all such further papers, including patent applications and instruments of transfer, and (c) perform such other acts as Assignee lawfully may request to obtain or maintain Letters Patent and Registrations for the invention and improvements in any and all countries, and to vest title thereto in Assignee, or Assignee's successors and assigns.

Assignors hereby authorize and request Townsend and Townsend and Crew LLP, Two Embarcadero Center, 8th Floor, San Francisco, CA 94111-3834, to insert herein above the application number and filing date of said application when known.

IN TESTIMONY WHEREOF, Assignors have signed their names on the dates indicated.

Dated: 5/15/01 — Alan J. Korman

Dated: 05-15-01 — Edward L. Halk

Dated: 05-15-01 — Nils Lonberg

Dated: 05-15-2001 — Yashwant M. Deo

Dated: 5/15/01 — Tibor P. Keler

PA 3108390 v1

3

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use YERVOY safely and effectively. See full prescribing information for YERVOY.

YERVOY™ (ipilimumab)
Injection, for intravenous infusion
Initial U.S. Approval: 2011

WARNING: IMMUNE-MEDIATED ADVERSE REACTIONS
See full prescribing information for complete boxed warning.

YERVOY can result in severe and fatal immune-mediated adverse reactions due to T-cell activation and proliferation. These immune-mediated reactions may involve any organ system; however, the most common severe immune-mediated adverse reactions are enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathy. The majority of these immune-mediated reactions initially manifested during treatment; however, a minority occurred weeks to months after discontinuation of YERVOY.

Permanently discontinue YERVOY and initiate systemic high-dose corticosteroid therapy for severe immune-mediated reactions. (2.2)

Assess patients for signs and symptoms of enterocolitis, dermatitis, neuropathy, and endocrinopathy and evaluate clinical chemistries including liver function tests and thyroid function tests at baseline and before each dose. (5.1, 5.2, 5.3, 5.4, 5.5)

INDICATIONS AND USAGE

YERVOY is a human cytotoxic T-lymphocyte antigen 4 (CTLA-4)-blocking antibody indicated for the treatment of unresectable or metastatic melanoma. (1)

DOSAGE AND ADMINISTRATION

- YERVOY 3 mg/kg administered intravenously over 90 minutes every 3 weeks for a total of four doses. (2.1)
- Permanently discontinue for severe adverse reactions. (2.2)

DOSAGE FORMS AND STRENGTHS

- 50 mg/10 mL (5 mg/mL) (3)
- 200 mg/40 mL (5 mg/mL) (3)

CONTRAINDICATIONS

None. (4)

WARNINGS AND PRECAUTIONS

Immune-mediated adverse reactions: Permanently discontinue for severe reactions. Withhold dose for moderate immune-mediated adverse reactions until return to baseline, improvement to mild severity, or complete resolution, and patient is receiving less than 7.5 mg prednisone or equivalent per day. Administer systemic high-dose corticosteroids for severe, persistent, or recurring immune-mediated reactions. (5.1, 5.2, 5.3, 5.4, 5.5)

- Immune-mediated hepatitis: Evaluate liver function tests before each dose of YERVOY.
- Immune-mediated endocrinopathies: Monitor thyroid function tests and clinical chemistries prior to each dose. Evaluate at each visit for signs and symptoms of endocrinopathy. Institute hormone replacement therapy as needed.

ADVERSE REACTIONS

Most common adverse reactions (≥5%) are fatigue, diarrhea, pruritus, rash, and colitis. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Bristol-Myers Squibb at 1-800-721-5072 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

USE IN SPECIFIC POPULATIONS

- Pregnancy: Based on animal data, YERVOY may cause fetal harm. (8.1)
- Nursing mothers: Discontinue nursing or discontinue YERVOY. (8.3)

See 17 for PATIENT COUNSELING INFORMATION and Medication Guide

Revised: March 2011

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed

FULL PRESCRIBING INFORMATION

WARNING: IMMUNE-MEDIATED ADVERSE REACTIONS

YERVOY can result in severe and fatal immune-mediated adverse reactions due to T-cell activation and proliferation. These immune-mediated reactions may involve any organ system; however, the most common severe immune-mediated adverse reactions are enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathy. The majority of these immune-mediated reactions initially manifested during treatment; however, a minority occurred weeks to months after discontinuation of YERVOY.

Permanently discontinue YERVOY and initiate systemic high-dose corticosteroid therapy for severe immune-mediated reactions. ***[See Dosage and Administration (2.2)]***

Assess patients for signs and symptoms of enterocolitis, dermatitis, neuropathy, and endocrinopathy and evaluate clinical chemistries including liver function tests and thyroid function tests at baseline and before each dose. ***[See Warnings and Precautions (5.1, 5.2, 5.3, 5.4, 5.5)]***

1 INDICATIONS AND USAGE

YERVOY (ipilimumab) is indicated for the treatment of unresectable or metastatic melanoma.

2 DOSAGE AND ADMINISTRATION

2.1 Recommended Dosing

The recommended dose of YERVOY is 3 mg/kg administered intravenously over 90 minutes every 3 weeks for a total of four doses.

2.2 Recommended Dose Modifications

- Withhold scheduled dose of YERVOY for any moderate immune-mediated adverse reactions or for symptomatic endocrinopathy. For patients with complete or partial resolution of adverse reactions (Grade 0–1), and who are receiving less than 7.5 mg prednisone or

equivalent per day, resume YERVOY at a dose of 3 mg/kg every 3 weeks until administration of all 4 planned doses or 16 weeks from first dose, whichever occurs earlier.

- Permanently discontinue YERVOY for any of the following:
  - Persistent moderate adverse reactions or inability to reduce corticosteroid dose to 7.5 mg prednisone or equivalent per day.
  - Failure to complete full treatment course within 16 weeks from administration of first dose.
  - Severe or life-threatening adverse reactions, including any of the following:
    - Colitis with abdominal pain, fever, ileus, or peritoneal signs; increase in stool frequency (7 or more over baseline), stool incontinence, need for intravenous hydration for more than 24 hours, gastrointestinal hemorrhage, and gastrointestinal perforation
    - Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >5 times the upper limit of normal or total bilirubin >3 times the upper limit of normal
    - Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations
    - Severe motor or sensory neuropathy, Guillain-Barré syndrome, or myasthenia gravis
    - Severe immune-mediated reactions involving any organ system (eg, nephritis, pneumonitis, pancreatitis, non-infectious myocarditis)
    - Immune-mediated ocular disease that is unresponsive to topical immunosuppressive therapy

2.3 Preparation and Administration

- Do not shake product.
- Inspect parenteral drug products visually for particulate matter and discoloration prior to administration. Discard vial if solution is cloudy, there is pronounced discoloration (solution may have pale yellow color), or there is foreign particulate matter other than translucent-to-white, amorphous particles.

Preparation of Solution

- Allow the vials to stand at room temperature for approximately 5 minutes prior to preparation of infusion.
- Withdraw the required volume of YERVOY and transfer into an intravenous bag.
- Dilute with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare a diluted solution with a final concentration ranging from 1 mg/mL to 2 mg/mL. Mix diluted solution by gentle inversion.
- Store the diluted solution for no more than 24 hours under refrigeration (2°C to 8°C, 36°F to 46°F) or at room temperature (20°C to 25°C, 68°F to 77°F).
- Discard partially used vials or empty vials of YERVOY.

Administration Instructions

- Do not mix YERVOY with, or administer as an infusion with, other medicinal products.
- Flush the intravenous line with 0.9% Sodium Chloride Injection, USP or 0.5% Dextrose Injection, USP after each dose.
- Administer diluted solution over 90 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein-binding in-line filter.

3 DOSAGE FORMS AND STRENGTHS

50 mg/10 mL (5 mg/mL).
200 mg/40 mL (5 mg/mL).

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

YERVOY can result in severe and fatal immune-mediated reactions due to T-cell activation and proliferation. *[See Boxed Warning]*

5.1 Immune-mediated Enterocolitis

In Study 1, severe, life-threatening, or fatal (diarrhea of 7 or more stools above baseline, fever, ileus, peritoneal signs; Grade 3–5) immune-mediated enterocolitis occurred in 34 (7%) YERVOY-treated patients, and moderate (diarrhea with up to 6 stools above baseline, abdominal pain, mucus or blood in stool; Grade 2) enterocolitis occurred in 28 (5%) YERVOY-treated patients. Across all YERVOY-treated patients (n=511), 5 (1%) patients developed intestinal perforation, 4 (0.8%) patients died as a result of complications, and 26 (5%) patients were hospitalized for severe enterocolitis.

The median time to onset was 7.4 weeks (range 1.6–13.4) and 6.3 weeks (range 0.3–18.9) after the initiation of YERVOY for patients with Grade 3–5 enterocolitis and with Grade 2 enterocolitis, respectively.

Twenty-nine patients (85%) with Grade 3–5 enterocolitis were treated with high-dose (≥40 mg prednisone equivalent per day) corticosteroids, with a median dose of 80 mg/day of prednisone or equivalent; the median duration of treatment was 2.3 weeks (ranging up to 13.9 weeks) followed by corticosteroid taper. Of the 28 patients with moderate enterocolitis, 46% were not treated with systemic corticosteroids, 29% were treated with <40 mg prednisone or equivalent per day for a median duration of 5.1 weeks, and 25% were treated with high-dose corticosteroids for a median duration of 10 days prior to corticosteroid taper. Infliximab was administered to 5 of the 62 patients (8%) with moderate, severe, or life-threatening immune-mediated enterocolitis following inadequate response to corticosteroids.

Of the 34 patients with Grade 3–5 enterocolitis, 74% experienced complete resolution, 3% experienced improvement to Grade 2 severity, and 24% did not improve. Among the 28 patients with Grade 2 enterocolitis, 79% experienced complete resolution, 11% improved, and 11% did not improve.

Monitor patients for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, mucus or blood in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). In symptomatic patients, rule out infectious etiologies and consider endoscopic evaluation for persistent or severe symptoms.

Permanently discontinue YERVOY in patients with severe enterocolitis and initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. Upon improvement to Grade 1 or less, initiate corticosteroid taper and continue to taper over at least one month. In

clinical trials, rapid corticosteroid tapering resulted in recurrence or worsening symptoms of enterocolitis in some patients.

Withhold YERVOY dosing for moderate enterocolitis; administer anti-diarrheal treatment and, if persistent for more than one week, initiate systemic corticosteroids at a dose of 0.5 mg/kg/day prednisone or equivalent. *[See Dosage and Administration (2.2)]*

5.2 Immune-mediated Hepatitis

In Study 1, severe, life-threatening, or fatal hepatotoxicity (AST or ALT elevations of more than 5 times the upper limit of normal or total bilirubin elevations more than 3 times the upper limit of normal; Grade 3–5) occurred in 8 (2%) YERVOY-treated patients, with fatal hepatic failure in 0.2% and hospitalization in 0.4% of YERVOY-treated patients. An additional 13 (2.5%) patients experienced moderate hepatotoxicity manifested by liver function test abnormalities (AST or ALT elevations of more than 2.5 times but not more than 5 times the upper limit of normal or total bilirubin elevation of more than 1.5 times but not more than 3 times the upper limit of normal; Grade 2). The underlying pathology was not ascertained in all patients but in some instances included immune-mediated hepatitis. There were insufficient numbers of patients with biopsy-proven hepatitis to characterize the clinical course of this event.

Monitor liver function tests (hepatic transaminase and bilirubin levels) and assess patients for signs and symptoms of hepatotoxicity before each dose of YERVOY. In patients with hepatotoxicity, rule out infectious or malignant causes and increase frequency of liver function test monitoring until resolution.

Permanently discontinue YERVOY in patients with Grade 3–5 hepatotoxicity and administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When liver function tests show sustained improvement or return to baseline, initiate corticosteroid tapering and continue to taper over 1 month. Across the clinical development program for YERVOY, mycophenolate treatment has been administered in patients who have persistent severe hepatitis despite high-dose corticosteroids. Withhold YERVOY in patients with Grade 2 hepatotoxicity. *[See Dosage and Administration (2.2)]*

5.3 Immune-mediated Dermatitis

In Study 1, severe, life-threatening, or fatal immune-mediated dermatitis (eg, Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations; Grade 3–5) occurred in 13 (2.5%)

YERVOY-treated patients. One (0.2%) patient died as a result of toxic epidermal necrolysis and one additional patient required hospitalization for severe dermatitis. There were 63 (12%) patients with moderate (Grade 2) dermatitis.

The median time to onset of moderate, severe, or life-threatening immune-mediated dermatitis was 3.1 weeks and ranged up to 17.3 weeks from the initiation of YERVOY.

Seven (54%) YERVOY-treated patients with severe dermatitis received high-dose corticosteroids (median dose 60 mg prednisone/day or equivalent) for up to 14.9 weeks followed by corticosteroid taper. Of these 7 patients, 6 had complete resolution; time to resolution ranged up to 15.6 weeks.

Of the 63 patients with moderate dermatitis, 25 (40%) were treated with systemic corticosteroids (median of 60 mg/day of prednisone or equivalent) for a median of 2.1 weeks, 7 (11%) were treated with only topical corticosteroids, and 31 (49%) did not receive systemic or topical corticosteroids. Forty-four (70%) patients with moderate dermatitis were reported to have complete resolution, 7 (11%) improved to mild (Grade 1) severity, and 12 (19%) had no reported improvement.

Monitor patients for signs and symptoms of dermatitis such as rash and pruritus. Unless an alternate etiology has been identified, signs or symptoms of dermatitis should be considered immune-mediated.

Permanently discontinue YERVOY in patients with Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations. Administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When dermatitis is controlled, corticosteroid tapering should occur over a period of at least 1 month. Withhold YERVOY dosing in patients with moderate to severe signs and symptoms. *[See Dosage and Administration (2.2)]*

For mild to moderate dermatitis, such as localized rash and pruritus, treat symptomatically. Administer topical or systemic corticosteroids if there is no improvement of symptoms within 1 week.

5.4 Immune-mediated Neuropathies

In Study 1, one case of fatal Guillain-Barré syndrome and one case of severe (Grade 3) peripheral motor neuropathy were reported. Across the clinical development program of

YERVOY, myasthenia gravis and additional cases of Guillain-Barré syndrome have been reported.

Monitor for symptoms of motor or sensory neuropathy such as unilateral or bilateral weakness, sensory alterations, or paresthesia. Permanently discontinue YERVOY in patients with severe neuropathy (interfering with daily activities) such as Guillain-Barré-like syndromes. Institute medical intervention as appropriate for management of severe neuropathy. Consider initiation of systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe neuropathies. Withhold YERVOY dosing in patients with moderate neuropathy (not interfering with daily activities). *[See Dosage and Administration (2.2)]*

5.5 Immune-mediated Endocrinopathies

In Study 1, severe to life-threatening immune-mediated endocrinopathies (requiring hospitalization, urgent medical intervention, or interfering with activities of daily living; Grade 3–4) occurred in 9 (1.8%) YERVOY-treated patients. All 9 patients had hypopituitarism and some had additional concomitant endocrinopathies such as adrenal insufficiency, hypogonadism, and hypothyroidism. Six of the 9 patients were hospitalized for severe endocrinopathies. Moderate endocrinopathy (requiring hormone replacement or medical intervention; Grade 2) occurred in 12 (2.3%) patients and consisted of hypothyroidism, adrenal insufficiency, hypopituitarism, and one case each of hyperthyroidism and Cushing's syndrome. The median time to onset of moderate to severe immune-mediated endocrinopathy was 11 weeks and ranged up to 19.3 weeks after the initiation of YERVOY.

Of the 21 patients with moderate to life-threatening endocrinopathy, 17 patients required long-term hormone replacement therapy including, most commonly, adrenal hormones (n=10) and thyroid hormones (n=13).

Monitor patients for clinical signs and symptoms of hypophysitis, adrenal insufficiency (including adrenal crisis), and hyper- or hypothyroidism. Patients may present with fatigue, headache, mental status changes, abdominal pain, unusual bowel habits, and hypotension, or nonspecific symptoms which may resemble other causes such as brain metastasis or underlying disease. Unless an alternate etiology has been identified, signs or symptoms of endocrinopathies should be considered immune-mediated.

Monitor thyroid function tests and clinical chemistries at the start of treatment, before each dose, and as clinically indicated based on symptoms. In a limited number of patients, hypophysitis was diagnosed by imaging studies through enlargement of the pituitary gland.

Withhold YERVOY dosing in symptomatic patients. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent, and initiate appropriate hormone replacement therapy. *[See Dosage and Administration (2.2)]*

5.6 Other Immune-mediated Adverse Reactions, Including Ocular Manifestations

The following clinically significant immune-mediated adverse reactions were seen in less than 1% of YERVOY-treated patients in Study 1: nephritis, pneumonitis, meningitis, pericarditis, uveitis, iritis, and hemolytic anemia.

Across the clinical development program for YERVOY, the following likely immune-mediated adverse reactions were also reported with less than 1% incidence: myocarditis, angiopathy, temporal arteritis, vasculitis, polymyalgia rheumatica, conjunctivitis, blepharitis, episcleritis, scleritis, leukocytoclastic vasculitis, erythema multiforme, psoriasis, pancreatitis, arthritis, and autoimmune thyroiditis.

Permanently discontinue YERVOY for clinically significant or severe immune-mediated adverse reactions. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe immune-mediated adverse reactions.

Administer corticosteroid eye drops to patients who develop uveitis, iritis, or episcleritis. Permanently discontinue YERVOY for immune-mediated ocular disease that is unresponsive to local immunosuppressive therapy. *[See Dosage and Administration (2.2)]*

6 ADVERSE REACTIONS

The following adverse reactions are discussed in greater detail in other sections of the labeling.

- Immune-mediated enterocolitis *[see Warnings and Precautions (5.1)]*.
- Immune-mediated hepatitis *[see Warnings and Precautions (5.2)]*.
- Immune-mediated dermatitis *[see Warnings and Precautions (5.3)]*.
- Immune-mediated neuropathies *[see Warnings and Precautions (5.4)]*.
- Immune-mediated endocrinopathies *[see Warnings and Precautions (5.5)]*.
- Other immune-mediated adverse reactions, including ocular manifestations *[see Warnings and Precautions (5.6)]*.

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, the adverse reaction rates observed cannot be directly compared with rates in other clinical trials or experience with therapeutics in the same class and may not reflect the rates observed in clinical practice.

The clinical development program excluded patients with active autoimmune disease or those receiving systemic immunosuppression for organ transplantation. Exposure to YERVOY 3 mg/kg for four doses given by intravenous infusion in previously treated patients with unresectable or metastatic melanoma was assessed in a randomized, double-blind clinical study (Study 1). *[See Clinical Studies (14)]* One hundred thirty-one patients (median age 57 years, 60% male) received YERVOY as a single agent, 380 patients (median age 56 years, 61% male) received YERVOY with an investigational gp100 peptide vaccine (gp100), and 132 patients (median age 57 years, 54% male) received gp100 peptide vaccine alone. Patients in the study received a median of 4 doses (range 1 to 4 doses). YERVOY was discontinued for adverse reactions in 10% of patients.

The most common adverse reactions (≥5%) in patients who received YERVOY at 3 mg/kg were fatigue, diarrhea, pruritus, rash, and colitis.

Table 1 presents selected adverse reactions from Study 1, which occurred in at least 5% of patients in the YERVOY-containing arms and with at least 5% increased incidence over the control gp100 arm for all-grade events and at least 1% incidence over the control group for Grade 3–5 events.

Table 1: Selected Adverse Reactions in Study 1

	Percentage (%) of Patients[a]					
	YERVOY 3 mg/kg n=131		YERVOY 3 mg/kg+gp100 n=380		gp100 n=132	
System Organ Class/ Preferred Term	Any Grade	Grade 3–5	Any Grade	Grade 3–5	Any Grade	Grade 3–5
Gastrointestinal Disorders						
Diarrhea	32	5	37	4	20	1
Colitis	8	5	5	3	2	0
Skin and Subcutaneous Tissue Disorders						
Pruritus	31	0	21	<1	11	0
Rash	29	2	25	2	8	0
General Disorders and Administration Site Conditions						
Fatigue	41	7	34	5	31	3

[a] Incidences presented in this table are based on reports of adverse events regardless of causality.

Table 2 presents the per-patient incidence of severe, life-threatening, or fatal immune-mediated adverse reactions from Study 1.

Table 2: Severe to Fatal Immune-mediated Adverse Reactions in Study 1

	Percentage (%) of Patients	
	YERVOY 3 mg/kg n=131	YERVOY 3 mg/kg+gp100 n=380
Any Immune-mediated Adverse Reaction	15	12
Enterocolitis[a,b]	7	7
Hepatotoxicity[a]	1	2
Dermatitis[a]	2	3
Neuropathy[a]	1	<1
Endocrinopathy	4	1
Hypopituitarism	4	1
Adrenal insufficiency	0	1
Other		
Pneumonitis	0	<1
Meningitis	0	<1
Nephritis	1	0
Eosinophilia[c]	1	0
Pericarditis[a,c]	0	<1

[a] Including fatal outcome.

[b] Including intestinal perforation.

[c] Underlying etiology not established.

Across clinical studies that utilized YERVOY doses ranging from 0.3 to 10 mg/kg, the following adverse reactions were also reported (incidence less than 1% unless otherwise noted): urticaria (2%), large intestinal ulcer, esophagitis, acute respiratory distress syndrome, renal failure, and infusion reaction.

Based on the experience in the entire clinical program for melanoma, the incidence and severity of enterocolitis and hepatitis appear to be dose dependent.

6.2 Immunogenicity

In clinical studies, 1.1% of 1024 evaluable patients tested positive for binding antibodies against ipilimumab in an electrochemiluminescent (ECL) based assay. This assay has substantial limitations in detecting anti-ipilimumab antibodies in the presence of ipilimumab. Infusion-related or peri-infusional reactions consistent with hypersensitivity or anaphylaxis were not reported in these 11 patients nor were neutralizing antibodies against ipilimumab detected.

Because trough levels of ipilimumab interfere with the ECL assay results, a subset analysis was performed in the dose cohort with the lowest trough levels. In this analysis, 6.9% of 58 evaluable patients, who were treated with 0.3 mg/kg dose, tested positive for binding antibodies against ipilimumab.

Immunogenicity assay results are highly dependent on several factors including assay sensitivity and specificity, assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of incidence of antibodies to YERVOY with the incidences of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

No formal drug-drug interaction studies have been conducted with YERVOY.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of YERVOY in pregnant women. Use YERVOY during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In a combined study of embryo-fetal and peri-postnatal development, severe toxicities including increased incidences of third-trimester abortion, stillbirth, premature delivery, low birth weight, and infant mortality occurred following intravenous administration of ipilimumab to pregnant cynomolgus monkeys every 21 days from the onset of organogenesis through parturition at doses of 2.6 or 7.2 times the recommended human dose of 3 mg/kg (by AUC). *[See Nonclinical Toxicology (13.2)]*

In genetically engineered mice in which the gene for CTLA-4 has been deleted (a "knockout mouse"), offspring lacking CTLA-4 were born apparently healthy, but died within 3–4 weeks due to multi-organ infiltration and damage by lymphocytes.

Human IgG1 is known to cross the placental barrier and ipilimumab is an IgG1; therefore, ipilimumab has the potential to be transmitted from the mother to the developing fetus.

8.3 Nursing Mothers

It is not known whether ipilimumab is secreted in human milk. Because many drugs are secreted in human milk and because of the potential for serious adverse reactions in nursing infants from YERVOY, a decision should be made whether to discontinue nursing or to discontinue YERVOY, taking into account the importance of YERVOY to the mother.

8.4 Pediatric Use

Safety and effectiveness of YERVOY have not been established in pediatric patients.

8.5 Geriatric Use

Of the 511 patients treated with YERVOY at 3 mg/kg, 28% were 65 years and over. No overall differences in safety or efficacy were reported between the elderly patients (65 years and over) and younger patients (less than 65 years).

8.6 Renal Impairment

No formal studies of YERVOY in patients with renal impairment have been conducted. *[See Clinical Pharmacology (12.3)]*

8.7 Hepatic Impairment

No formal studies of YERVOY in patients with hepatic impairment have been conducted. *[See Clinical Pharmacology (12.3)]*

10 OVERDOSAGE

There is no information on overdosage with YERVOY.

11 DESCRIPTION

YERVOY (ipilimumab) is a recombinant, human monoclonal antibody that binds to the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). Ipilimumab is an IgG1 kappa immunoglobulin with an approximate molecular weight of 148 kDa. Ipilimumab is produced in mammalian (Chinese hamster ovary) cell culture.

YERVOY is a sterile, preservative-free, clear to slightly opalescent, colorless to pale yellow solution for intravenous infusion, which may contain a small amount of visible translucent-to-white, amorphous ipilimumab particulates. It is supplied in single-use vials of 50 mg/10 mL and 200 mg/40 mL. Each milliliter contains 5 mg of ipilimumab and the following inactive ingredients: diethylene triamine pentaacetic acid (DTPA) (0.04 mg), mannitol (10 mg), polysorbate 80 (vegetable origin) (0.1 mg), sodium chloride (5.85 mg), tris hydrochloride (3.15 mg), and Water for Injection, USP at a pH of 7.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

CTLA-4 is a negative regulator of T-cell activation. Ipilimumab binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation. The mechanism of action of ipilimumab's effect in patients with melanoma is indirect, possibly through T-cell mediated anti-tumor immune responses.

12.3 Pharmacokinetics

The pharmacokinetics of ipilimumab was studied in 499 patients with unresectable or metastatic melanoma who received doses of 0.3, 3, or 10 mg/kg administered once every 3 weeks for four doses. Peak concentration (C_{max}), trough concentration (C_{min}), and area under the curve (AUC) of ipilimumab were found to be dose proportional within the dose range examined. Upon repeated dosing of YERVOY administered every 3 weeks, ipilimumab clearance was found to be time-invariant, and minimal systemic accumulation was observed as evident by an accumulation index of 1.5-fold or less. Ipilimumab steady-state concentration was reached by the third dose. The following mean (percent coefficient of variation) parameters were generated through population pharmacokinetic analysis: terminal half-life of 14.7 days (30.1%); systemic clearance (CL) of 15.3 mL/h (38.5%); and volume of distribution at steady-state (Vss) of 7.21 L (10.5%). The

mean (±SD) ipilimumab C_{min} achieved at steady-state with the 3-mg/kg regimen was 21.8 mcg/mL (±11.2).

Specific Populations: Cross-study analyses were performed on data from patients with a variety of conditions, including 420 patients with melanoma who received single or multiple infusions of YERVOY at doses of 0.3, 3, or 10 mg/kg. The effects of various covariates on ipilimumab pharmacokinetics were assessed in population pharmacokinetic analyses.

Ipilimumab CL increased with increasing body weight; however, no dose adjustment of YERVOY is required for body weight after administration on a mg/kg basis. The following factors had no clinically meaningful effect on the CL of ipilimumab: age (range 26 to 86 years), gender, concomitant use of budesonide, performance status, HLA-A2*0201 status, positive anti-ipilimumab antibody status, prior use of systemic anticancer therapy, or baseline lactate dehydrogenase (LDH) levels. The effect of race was not examined as there were insufficient numbers of patients in non-Caucasian ethnic groups.

Renal Impairment: Creatinine clearance at baseline did not have a clinically important effect on ipilimumab pharmacokinetics in patients with calculated creatinine clearance values of 29 mL/min or greater.

Hepatic Impairment: Baseline AST, total bilirubin, and ALT levels did not have a clinically important effect on ipilimumab pharmacokinetics in patients with various degrees of hepatic impairment.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

The carcinogenic potential of ipilimumab has not been evaluated in long-term animal studies.

Mutagenesis

The genotoxic potential of ipilimumab has not been evaluated.

Impairment of Fertility

Fertility studies have not been performed with ipilimumab.

13.2 Animal Toxicology and/or Pharmacology

The effects of ipilimumab on prenatal and postnatal development in monkeys have not been fully investigated. Preliminary results are available from an ongoing study in cynomolgus monkeys. Pregnant monkeys received ipilimumab every 21 days from the onset of organogenesis in the first trimester through delivery, at dose levels either 2.6 or 7.2 times higher than the clinical dose of 3 mg/kg of ipilimumab (by AUC). No treatment-related adverse effects on reproduction were detected during the first two trimesters of pregnancy. Beginning in the third trimester, the ipilimumab groups experienced higher incidences of abortion, stillbirth, premature delivery (with corresponding lower birth weight), and higher incidences of infant mortality in a dose-related manner compared to controls.

Genetically engineered mice heterozygous for CTLA-4 (CTLA-4+/-), the target for ipilimumab, appeared healthy and gave birth to healthy CTLA-4+/- heterozygous offspring. Mated CTLA-4+/- heterozygous mice also produced offspring deficient in CTLA-4 (homozygous negative, CTLA-4-/-). The CTLA-4-/- homozygous negative offspring appeared healthy at birth, exhibited signs of multiorgan lymphoproliferative disease by 2 weeks of age, and all died by 3–4 weeks of age with massive lymphoproliferation and multiorgan tissue destruction.

14 CLINICAL STUDIES

The safety and efficacy of YERVOY were investigated in a randomized (3:1:1), double-blind, double-dummy study (Study 1) that included 676 randomized patients with unresectable or metastatic melanoma previously treated with one or more of the following: aldesleukin, dacarbazine, temozolomide, fotemustine, or carboplatin. Of these 676 patients, 403 were randomized to receive YERVOY at 3 mg/kg in combination with an investigational peptide vaccine with incomplete Freund's adjuvant (gp100), 137 were randomized to receive YERVOY at 3 mg/kg, and 136 were randomized to receive gp100 alone. The study enrolled only patients with HLA-A2*0201 genotype; this HLA genotype facilitates the immune presentation of the investigational peptide vaccine. The study excluded patients with active autoimmune disease or those receiving systemic immunosuppression for organ transplantation. YERVOY/placebo was administered at 3 mg/kg as an intravenous infusion every 3 weeks for four doses. Gp100/placebo was administered at a dose of 2 mg peptide by deep subcutaneous injection every 3 weeks for four doses. Assessment of tumor response was conducted at weeks 12 and 24, and every 3 months thereafter. Patients with evidence of objective tumor response at 12 or 24 weeks had assessment for confirmation of durability of response at 16 or 28 weeks, respectively.

The major efficacy outcome measure was overall survival (OS) in the YERVOY+gp100 arm compared to that in the gp100 arm. Secondary efficacy outcome measures were OS in the YERVOY+gp100 arm compared to the YERVOY arm, OS in the YERVOY arm compared to the gp100 arm, best overall response rate (BORR) at week 24 between each of the study arms, and duration of response.

Of the randomized patients, 61%, 59%, and 54% in the YERVOY+gp100, YERVOY, and gp100 arms, respectively, were men. Twenty-nine percent were ≥65 years of age, the median age was 57 years, 71% had M1c stage, 12% had a history of previously treated brain metastasis, 98% had ECOG performance status of 0 and 1, 23% had received aldesleukin and 38% had elevated LDH level. Sixty-one percent of patients randomized to either YERVOY-containing arm received all 4 planned doses. The median duration of follow-up was 8.9 months.

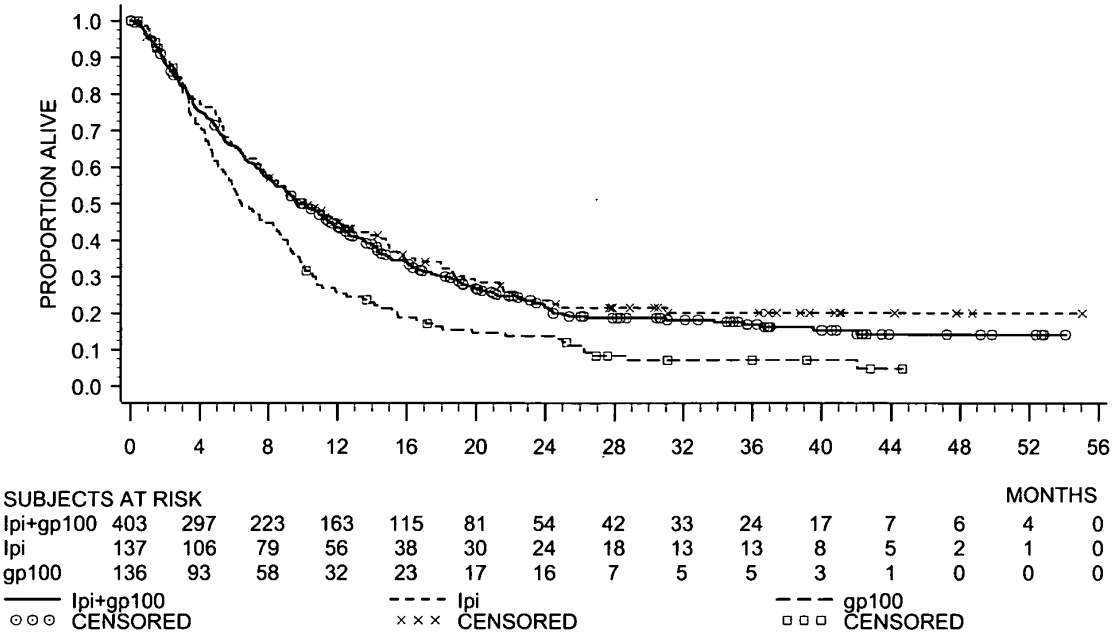
The OS results are shown in Table 3 and Figure 1.

Table 3: Overall Survival Results

	YERVOY n=137	YERVOY+gp100 n=403	gp100 n=136
Hazard Ratio (vs. gp100) (95% CI)	0.66 (0.51, 0.87)	0.68 (0.55, 0.85)	
p-value	p=0.0026[a]	p=0.0004	
Hazard Ratio (vs. YERVOY) (95% CI)		1.04 (0.83, 1.30)	
Median (months) (95% CI)	10 (8.0, 13.8)	10 (8.5, 11.5)	6 (5.5, 8.7)

[a] Not adjusted for multiple comparisons.

Figure 1: Overall Survival



The best overall response rate (BORR) as assessed by the investigator was 5.7% (95% CI: 3.7%, 8.4%) in the YERVOY+gp100 arm, 10.9% (95% CI: 6.3%, 17.4%) in the YERVOY arm, and 1.5% (95% CI: 0.2%, 5.2%) in the gp100 arm. The median duration of response was 11.5 months in the YERVOY+gp100 arm and has not been reached in the YERVOY or gp100 arm.

16 HOW SUPPLIED/STORAGE AND HANDLING

YERVOY is available as follows:

Carton Contents	NDC
One 50 mg vial (5 mg/mL), single-use vial	NDC 0003-2327-11
One 200 mg vial (5 mg/mL), single-use vial	NDC 0003-2328-22

Store YERVOY under refrigeration at 2°C to 8°C (36°F to 46°F). Do not freeze. Protect vials from light.

17 PATIENT COUNSELING INFORMATION

See MEDICATION GUIDE.

- Inform patients of the potential risk of immune-mediated adverse reactions.
- Advise patients to read the YERVOY Medication Guide before each YERVOY infusion.
- Advise women that YERVOY may cause fetal harm.
- Advise nursing mothers not to breast-feed while taking YERVOY.

Manufactured by: Bristol-Myers Squibb Company
Princeton, NJ 08543 USA
U.S. License No. 1713

MEDICATION GUIDE

YERVOY™ (yur-voi)

(ipilimumab)

Read this Medication Guide before you start receiving YERVOY and before each infusion. There may be new information. This Medication Guide does not take the place of talking with your healthcare provider about your medical condition or your treatment.

What is the most important information I should know about YERVOY?

YERVOY can cause serious side effects in many parts of your body which can lead to death. These side effects are most likely to begin during treatment; however, side effects can show up months after your last infusion.

These side effects may include:

1. **Inflammation of the intestines (colitis) that can cause tears or holes (perforation) in the intestines.** Signs and symptoms of colitis may include:
   - diarrhea (loose stools) or more bowel movements than usual
   - blood in your stools or dark, tarry, sticky stools
   - stomach pain (abdominal pain) or tenderness

2. **Inflammation of the liver (hepatitis) that can lead to liver failure.** Signs and symptoms of hepatitis may include:
   - yellowing of your skin or the whites of your eyes
   - dark urine (tea colored)
   - nausea or vomiting
   - pain on the right side of your stomach
   - bleeding or bruise more easily than normal

3. **Inflammation of the skin that can lead to severe skin reaction (toxic epidermal necrolysis).** Signs and symptoms of severe skin reactions may include:
   - skin rash with or without itching
   - sores in your mouth
   - your skin blisters and/or peels

4. **Inflammation of the nerves that can lead to paralysis.** Symptoms of nerve problems may include:
   - unusual weakness of legs, arms, or face
   - numbness or tingling in hands or feet

5. **Inflammation of hormone glands (especially the pituitary, adrenal, and thyroid glands) that may affect how these glands work.** Signs and symptoms that your glands are not working properly may include:
   - persistent or unusual headaches
   - unusual sluggishness, feeling cold all the time, or weight gain
   - changes in mood or behavior such as decreased sex drive, irritability, or forgetfulness
   - dizziness or fainting

6. **Inflammation of the eyes.** Symptoms may include:
   - blurry vision, double vision, or other vision problems
   - eye pain or redness

Call your healthcare provider if you have any of these signs or symptoms or they get worse. Do not try to treat symptoms yourself.

Getting medical treatment right away may keep the problem from becoming more serious. Your oncologist may decide to delay or stop YERVOY.

What is YERVOY?

YERVOY is a prescription medicine used in adults to treat melanoma (a kind of skin cancer) that has spread or cannot be removed by surgery.

It is not known if YERVOY is safe and effective in children less than 18 years of age.

What should I tell my healthcare provider before getting YERVOY?

Before you are given YERVOY, tell your healthcare provider about all your health problems if you:
- have an active condition where your immune system attacks your body (autoimmune disease), such as ulcerative colitis, Crohn's disease, lupus, or sarcoidosis
- had an organ transplant, such as a kidney transplant
- have liver damage from diseases or drugs
- have any other medical conditions
- are pregnant or plan to become pregnant. YERVOY may cause stillbirth, premature delivery, and/or death of your unborn baby
- are breast-feeding

Tell your healthcare provider about all the medicines you take, including all prescription and non-prescription medicines, steroids or other medicines that lower your immune response, vitamins, and herbal supplements.

Know the medicines you take. Keep a list to show your doctors and pharmacists each time you get a new medicine.

You should not start a new medicine before your talk with the healthcare provider who prescribes you YERVOY.

How will I receive YERVOY?

You will get YERVOY through an intravenous line in your vein (infusion). It takes about 90 minutes to get a full dose.

- YERVOY is usually given every 3 weeks for up to 4 doses. Your healthcare provider may change how often you receive YERVOY or how long the infusion may take.
- Your healthcare provider should perform blood tests before starting and during treatment with YERVOY.

It is important for you to keep all appointments with your healthcare provider. Call your healthcare provider if you miss an appointment. There may be special instructions for you.

What are the possible side effects of YERVOY?

YERVOY can cause serious side effects. See "**What is the most important information I should know about YERVOY?**"

The most common side effects of YERVOY include:

- tiredness
- diarrhea
- itching
- rash

These are not all of the possible side effects of YERVOY. For more information, ask your healthcare provider.

Call your healthcare provider for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

You may also report side effects to Bristol-Myers Squibb at 1-800-721-5072.

General information about the safe and effective use of YERVOY.

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide.

This Medication Guide summarizes the most important information about YERVOY. If you would like more information, talk with your healthcare provider. You can ask your healthcare provider for information about YERVOY that is written for healthcare professionals.

For more information, call 1-800-321-1335.

What are the ingredients of YERVOY?

Active ingredient: ipilimumab

Inactive ingredients: diethylene triamine pentaacetic acid (DTPA), mannitol, polysorbate 80, sodium chloride, tris hydrochloride, and Water for Injection, USP

This Medication Guide has been approved by the U.S. Food and Drug Administration.

Manufactured by: Bristol-Myers Squibb Company
Princeton, NJ 08543 USA
U.S. License Number 1713

Bristol-Myers Squibb Company
Princeton, NJ 08543 USA

1281558 Issued: March 2011

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

Our STN: BL 125377/0

BLA APPROVAL
March 25, 2011

Bristol-Myers Squibb Company
Attention: A. Heather Knight-Trent, PharmD
Director-Oncology
5 Research Parkway
Wallingford, CT 06492-7660

Dear Dr. Knight-Trent:

Please refer to your Biologics License Application (BLA) dated June 25, 2010, received June 25, 2010, submitted under section 351 of the Public Health Service Act for YERVOY (ipilimumab).

We acknowledge receipt of all subsequent amendments received through March 24, 2011.

We have approved your BLA for ipilimumab effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, ipilimumab, under your existing Department of Health and Human Services U.S. License No. 1713. Ipilimumab is indicated for the treatment of unresectable or metastatic melanoma.

Under this license, you are approved to manufacture ipilimumab drug substance at Lonza Biologics, Incorporated at Portsmouth, New Hampshire. The final formulated product will be manufactured, filled, labeled and packaged at Baxter Pharmaceutical Solutions, LLC at Bloomington, Indiana. You may label your product with the proprietary name YERVOY and will market it in 50 mg/10 mL and 200 mg/40 mL single-use vials.

Your application for ipilimumab was not referred to an FDA advisory committee because outside expertise was not necessary; there were no controversial issues that would benefit from advisory committee discussion.

The dating period for ipilimumab shall be 36 months from the date of manufacture when stored at 2-8 °C, but should not exceed 48 months from the date of drug substance manufacture. The date of drug product manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for your drug substance shall be 36 months from the date of manufacture when stored at 2-8 °C. The expiration date for the packaged product, ipilimumab single-use vials, shall be dependent on the shortest expiration date of any component.

We have approved the stability protocols in your license application for the purpose of extending the expiration dating period of your drug substance and drug product under 21 CFR 601.12.

You are not currently required to submit samples of future lots of ipilimumab to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1, requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

Any changes in the manufacturing, testing, packaging, or labeling of ipilimumab, or in the manufacturing facilities, will require the submission of information to your biologics license application for our review and written approval, consistent with 21 CFR 601.12.

We are approving this application for use as recommended in the enclosed agreed-upon labeling text.

CONTENT OF LABELING

As soon as possible, but no later than 14 days from the date of this letter, submit, via the FDA automated drug registration and listing system (eLIST), the content of labeling [21 601.14(b)] in structured product labeling (SPL) format, as described at http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm, that is identical to the enclosed labeling (text for the package insert, Medication Guide). Information on submitting SPL files using eLIST may be found in the guidance for industry titled "SPL Standard for Content of Labeling Technical Qs and As" at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf. For administrative purposes, please designate this submission "**Product Correspondence – Final SPL for approved BLA STN 125377/0.**"

The SPL will be accessible via publicly available labeling repositories.

CARTON AND IMMEDIATE CONTAINER LABELS

Submit final printed carton and container labels that are identical to the enclosed carton and immediate container labels and carton and immediate container labels submitted on March 11, 2011 as soon as they are available, but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled "Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008)". Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission "**Product Correspondence – Final Printed Carton and Container Labels for approved BLA STN 125377/0.**" Approval of this submission by FDA is not required before the labeling is used.

Marketing the product with final printed labeling (FPL) that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication in pediatric patients unless this requirement is waived, deferred, or inapplicable.

Because this drug product for this indication has an orphan drug designation, you are exempt from this requirement.

POSTMARKETING REQUIREMENTS UNDER 505(o)

Section 505(o)(3) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute.

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to identify an unexpected serious risk of embryo-fetal toxicity or anti-drug antibody responses.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA is not yet sufficient to assess these serious risks.

Therefore, based on appropriate scientific data, FDA has determined that you are required to conduct the following:

1. To submit the final report for study DN120020 (Intravenous Study of Pre- and Post-natal Developmental in Cynomolgus Monkeys with a 6-Month Post-natal Evaluation).

   The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

   Final Report Submission: December 31, 2011

2. To develop a validated, sensitive, and accurate assay for the detection of binding antibodies to ipilimumab, including procedures for accurate detection of antibodies to ipilimumab in the presence of ipilimumab levels that are expected to be present in the serum or plasma at the time of patient sampling.

   The timetable you submitted on March 14, 2011, states that you will conduct this assay according to the following schedule:

   Final Report Submission (Assay and Methodology): December 2, 2011

3. To develop a validated, sensitive, and accurate assay for the detection of neutralizing antibodies to ipilimumab, including procedures for accurate detection of neutralizing antibodies to ipilimumab in the presence of ipilimumab levels that are expected to be present in the serum or plasma at the time of patient sampling. In the event such an assay can not be developed, evidence of due diligence in attempting to develop the assay will be provided.

   The timetable you submitted on March 14, 2011, states that you will conduct this assay according to the following schedule:

   Final Report Submission (Assay and Methodology): February 20, 2012

Finally, we have determined that only a clinical trial (rather than a nonclinical or observational study) will be sufficient to address the following:

- Identify unexpected serious risk of anti-drug antibody responses;
- Assess a signal of serious risk of immune-mediated adverse reactions associated with CD86 gene polymorphisms;
- Assess a known serious risk of fatal and life-threatening immune-mediated adverse reactions

Therefore, based on appropriate scientific data, FDA has determined that you are required, to conduct the following:

4. To conduct an assessment of anti-drug antibody (ADA) response and neutralizing ADA responses to ipilimumab with a validated assay (required in PMR 2 and 3) capable of sensitively detecting ADA responses in the presence of ipilimumab levels that are expected to be present at the time of patient sampling. The ADA response will be evaluated in at least 300 ipilimumab-treated patients enrolled in the required postmarketing trial (PMR 6) comparing 3 mg/kg versus 10 mg/kg of ipilimumab monotherapy. The final report will include information on the level of ipilimumab in each patient's test sample at each sampling time point.

   The timetable you submitted on March 14, 2011, states that you will conduct this assessment from clinical trial data according to the following schedule:

Final Protocol Submission:	September 30, 2011
Patient Accrual Completed	December 31, 2014
Trial Completion Date:	August 31, 2017
Final Report Submission:	December 29, 2017

5. During the conduct of the required postmarketing trial comparing 3mg/kg vs. 10mg/kg ipilimumab monotherapy (PMR 6), you will obtain comprehensive baseline DNA sample acquisition (≥95% of ITT) and conduct pharmacogenomic association analyses to assess the potential clinical utility of CD86 gene polymorphisms as genetic determinants of immune mediated adverse events. You will provide a protocol that addresses SNP selection, data analyses approaches, and other methodological issues. You will provide a Final Report including electronic datasets.

   The timetable you submitted on March 14, 2011, states that you will conduct this assessment from clinical trial data according to the following schedule:

Draft Protocol Submission:	November 30, 2011
Final Protocol Submission:	May 30, 2012
Final Report Submission:	December 29, 2016

6. Following the assessment of data from Trial CA184024, you will design and conduct a trial to compare the efficacy, with the primary endpoint of overall survival and the safety of ipilimumab at doses of 3mg/kg versus 10mg/kg given as monotherapy every three weeks for four doses in patients with unresectable Stage III or Stage IV melanoma.

   The timetable you submitted on March 14, 2011, states that you will conduct this trial according to the following schedule:

Preliminary CA184024 Data Submission:	June 30, 2011
Draft Protocol Synopsis Submission:	June 30, 2011
Final Protocol Submission:	September 30, 2011
First Patient Accrued to Trial:	March 30, 2012
Last Patient Accrued to Trial:	December 31, 2014
Trial Completion:	August, 31, 2017
Final Report Submission:	December 31, 2017

Submit protocols to your IND, with a cross-reference letter to this BLA. Submit all final reports to your BLA. Prominently identify the submission with the following wording in bold capital letters at the top of the first page of the submission, as appropriate:

- **REQUIRED POSTMARKETING PROTOCOL UNDER 505(o)**
- **REQUIRED POSTMARKETING FINAL REPORT UNDER 505(o)**
- **REQUIRED POSTMARKETING CORRESPONDENCE UNDER 505(o)**

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 601.70 requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 601.70 to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 601.70. We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

POSTMARKETING COMMITMENTS SUBJECT TO THE REPORTING REQUIREMENTS UNDER SECTION 506B

We remind you of your postmarketing commitments:

7. To identify further genetic determinants of immune-mediated adverse events caused by ipilimumab. DNA samples from the required postmarketing study comparing 3 mg/kg vs. 10 mg/kg ipilimumab monotherapy will be used to conduct genome-wide association analyses. The design of these analyses will be reviewed by FDA and a final report with electronic datasets will be provided.

   The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

Draft Protocol Submission:	December 29, 2016
Final Protocol Submission:	July 31, 2017
Final Report Submission:	December 31, 2018

POSTMARKETING COMMITMENTS NOT SUBJECT TO THE REPORTING REQUIREMENTS UNDER SECTION 506B

We remind you of your postmarketing commitments:

8. To develop and validate a semi-quantitative assay to evaluate visible particulates in drug product. The assay will be incorporated into the drug product release and stability testing programs. The final validation report with the specifications and method validation will be submitted as a CBE-30 supplement by May 30, 2011.

   The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

   Final Report Submission as a CBE-30 supplement: May 30, 2011

9. To replace the IEF assay with the CEX assay for the release of drug product after sufficient data has been acquired to support establishment of CEX acceptance criteria. The final study report will be submitted as a CBE-30 by June 30, 2011.

   The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

   Final Report Submission as a CBE-30 supplement: June 30, 2011

10. To discontinue the IEF method as a specification for charge in the drug substance and drug product stability programs after three years of market life data are collected for the CEX assay on three batches of drug substance and three batches of either presentation of drug product. The final results and proposed CEX specification will be submitted as a CBE-30 supplement by March 31, 2014.

    The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

    Final Report Submission as a CBE-30 supplement: March 31, 2014

11. To perform studies to confirm that clearance of Antifoam C is well controlled by the manufacturing process and provide a risk assessment for residual amounts that may be present in the drug product. The final report will be submitted as a CBE-0 supplement by July 29, 2011.

    The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

    Final Report Submission as a CBE-30 supplement: July 29, 2011

12. To develop and validate a process-specific host cell protein (HCP) ELISA. This assay will replace the current Cygnus Kit ELISA being used in the drug substance release program. The final study and validation reports will be submitted as a CBE-30 supplement by November 30, 2011.

    The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

    Final Report/Validation Report Submission
    as a CBE-30 supplement: November 30, 2011

13. To reassess release and stability specifications for ipilimumab drug substance and drug product through April 30, 2013. The assessment will be submitted in the 2013 Annual Report.

    The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

    Final Report Submission (Annual Report): May 2013

14. To submit the final study reports for studies performed to confirm product stability over the course of the in-process hold times of 14 days at 2-8°C and 72 hours at 22-28°C. Final study results will be submitted in the 2012 Annual Report.

    The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

    Final Report Submission (Annual Report): May 2012

15. To submit the final concurrent column life-time study reports for the Poros 50HS, Q-Sepharose and CHT Type II columns. The final report will be submitted in the 2013 Annual Report.

    The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

    Final Report Submission (Annual Report): May 2013

16. To submit the final study reports for the drug substance storage container leachate studies to assess the volatile organic compounds (VOC), semi-VOC, non-VOC and trace metals in drug substance and formulation buffer samples held at 2 to 8°C for up to 3 years and under accelerated aging conditions of 40°C to simulate 3 years at 2 to 8°C. Final reports will be submitted in the 2013 Annual Report.

    The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

    Final Report Submission (Annual Report): May 2013

17. To re-assess the bioburden action limits for the purification in-process intermediates based on the manufacturing scale data from 30 lots using a 10 mL sample volume and submit the summary report in a CBE-0 supplement by March 31, 2013.

The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

Final Report Submission as a CBE-0 supplement: March 31, 2013

18. To develop and implement a container closure integrity test to replace the sterility test in the stability program. The ability of a container closure system to maintain the integrity of its microbial barrier and hence the sterility of a drug product throughout its shelf-life should be demonstrated. Submit the summary report and data in a CBE-0 supplement by December 2011.

The timetable you submitted on March 14, 2011, states that you will conduct this study according to the following schedule:

Final Report Submission as a CBE-0 supplement: December 31, 2011

Submit clinical protocols to your IND 9186 for this product. Submit nonclinical and chemistry, manufacturing, and controls protocols and all final reports to this BLA. In addition, under 21 CFR 601.70 you should include a status summary of each commitment in your annual progress report of postmarketing studies to this BLA. The status summary should include expected summary completion and final report submission dates, any changes in plans since the last annual report, and, for clinical studies/trials, number of patients entered into each study/trial. All submissions, including supplements, relating to these postmarketing commitments should be prominently labeled **"Postmarketing Commitment Protocol," "Postmarketing Commitment Final Report,"** or **"Postmarketing Commitment Correspondence."**

RISK EVALUATION AND MITIGATION STRATEGY REQUIREMENTS

Section 505-1 of the FDCA authorizes FDA to require the submission of a risk evaluation and mitigation strategy (REMS), if FDA determines that such a strategy is necessary to ensure that the benefits of the drug outweigh the risks (section 505-1(a)).

In accordance with section 505-1 of FDCA, we have determined that a REMS is necessary for YERVOY (ipilimumab) to ensure the benefits of the drug outweigh the risks of severe and fatal immune-mediated adverse reactions such as fatal immune-mediated enterocolitis (including gastrointestinal perforation), fatal immune-mediated hepatitis (including hepatic failure), fatal immune-mediated toxicities of the skin (including toxic epidermal necrolysis), fatal nervous system toxicity, and endocrinopathies, associated with the use of YERVOY (ipilimumab).

We have determined that a communication plan targeted to healthcare providers is necessary to support implementation of the REMS.

Your proposed REMS, submitted on June 25, 2010, as amended, and appended to this letter, is approved. The REMS consists of a communication plan and a timetable for submission of assessments of the REMS.
The REMS assessment plan should include but is not limited to the following:

a. An evaluation of healthcare providers' (HCPs) understanding of the serious risks of YERVOY (ipilimumab) and the management of the immune-mediated adverse reactions caused by YERVOY.

b. With regard to assessment of the communication plan:
   i The date of product launch and the launch of the communication plan.
   ii The date(s) of mailing and number of recipients of the Dear Healthcare Provider (DHCP) letter and the communication package.
   iii The number of mailings returned.
   iv The sources of the recipient lists.
   v The number of new prescribers prescribing YERVOY (ipilimumab) /new facilities purchasing YERVOY (ipilimumab) during the reporting period. Of the new prescribers/purchasers, the number supplied with the communication materials within the required timeframe; the number not supplied with communication materials within the required timeframe; the reasons for the failure to deliver communication materials within the required timeframe.

c. Based on the information submitted, an assessment of and conclusion regarding whether the REMS is meeting its goals, and whether modifications to the REMS are needed.

d. Specification of measures that would be taken to increase awareness if surveys of HCPs indicate that provider awareness is not adequate.

e. An analysis of post-marketing cases of immune-mediated adverse events reported for YERVOY that result in the patient's death, including an analysis of the length and reasons for any reported delay in recognition and treatment of the events.

f. Information on the status of any post-approval study or clinical trial required under section 505(o) or otherwise undertaken to investigate a safety issue. With respect to any such post-approval study, you must include the status of such study, including whether any difficulties completing the study have been encountered. With respect to any such post-approval clinical trial, you must include the status of such clinical trial, including whether enrollment has begun, the number of participants enrolled, the expected completion date, whether any difficulties completing the clinical trial have been encountered, and registration information with respect to requirements under subsections (i) and (j) of section 402 of the Public Health Service Act. You can satisfy these requirements in your REMS assessments by referring to relevant information included in the most recent annual report required under section 506B and 21 CFR 601.70 and including any material or significant updates to the status information since the annual report was prepared. Failure to comply with the REMS assessments provisions in section 505-1(g) could result in enforcement action.

Submit the methodology and survey instrument(s) for review at least 90 days before the next evaluation is conducted. Submit both methods and instruments together.

We remind you that in addition to the assessments submitted according to the timetable included in the approved REMS, you must submit a REMS assessment and may propose a modification to the approved REMS when you submit a supplemental application for a new indication for use as described in section 505-1(g)(2)(A) of the FDCA.

Prominently identify the submission containing the REMS assessments or proposed modifications with the following wording in bold capital letters at the top of the first page of the submission:

BLA 125377
REMS ASSESSMENT

NEW SUPPLEMENT FOR BLA 125377
PROPOSED REMS MODIFICATION
REMS ASSESSMENT

NEW SUPPLEMENT (NEW INDICATION FOR USE)
FOR BLA 125377
REMS ASSESSMENT
PROPOSED REMS MODIFICATION (if included)

If you do not submit electronically, please send 5 copies of REMS-related submissions.

REPORTING REQUIREMENTS

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to:

Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
5901-B Ammendale Road
Beltsville, MD 20705-1266

Biological product deviations, sent by courier or overnight mail, should be addressed to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
10903 New Hampshire Avenue, Bldg. 51, Room 4206
Silver Spring, MD 20903

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications
5901-B Ammendale Road
Beltsville, MD 20705-1266

You must submit final promotional materials, and the package insert, at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Division of Drug Marketing, Advertising, and Communications (DDMAC), see http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

LETTERS TO HEALTH CARE PROFESSIONALS

We acknowledge that you will issue a letter communicating important safety-related information about this drug product (i.e., a "Dear Health Care Professional" letter); we request that you submit, at least 24 hours prior to issuing the letter, an electronic copy of the letter to this BLA to the following address:

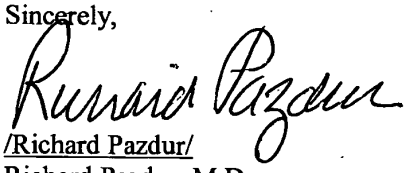
MedWatch Program
Office of Special Health Issues
Food and Drug Administration
10903 New Hampshire Ave
Building 32, Mail Stop 5353
Silver Spring, MD 20993

POST-ACTION FEEDBACK MEETING

New molecular entities and new biologics qualify for a post-action feedback meeting. Such meetings are used to discuss the quality of the application and to evaluate the communication process during drug development and marketing application review. The purpose is to learn from successful aspects of the review process and to identify areas that could benefit from improvement. If you would like to have such a meeting with us, call the Regulatory Project Manager for this application.

If you have any questions, call Erik S. Laughner, M.S., RAC (US), Senior Regulatory Health Project Manager, at (301) 796-1393.

Sincerely,



/Richard Pazdur/
Richard Pazdur, M.D.
Director,
Office of Oncology Drug Products
Center for Drug Evaluation and Research

ENCLOSURES:
Content of Labeling
Carton and Container Labeling
REMS
REMS Materials

5

Return To:

USPTO Home Page

Finance Online Shopping Page

United States Patent and Trademark Office

Patent Bibliographic Data				05/13/2011 04:22 PM	
Patent Number:	6984720		**Application Number:**	09644668	
Issue Date:	01/10/2006		**Filing Date:**	08/24/2000	
Title:	HUMAN CTLA-4 ANTIBODIES				
Status:	8th year fee window opens: 01/10/2013			**Entity:**	Large
Window Opens:	01/10/2013	**Surcharge Date:**	07/11/2013	**Expiration:**	N/A
Fee Amt Due:	Window not open	**Surchg Amt Due:**	Window not open	**Total Amt Due:**	Window not open
Fee Code:	1552	MAINTENANCE FEE DUE AT 7.5 YEARS			
Surcharge Fee Code:					
Most recent events (up to 7):	07/10/2009	Payment of Maintenance Fee, 4th Year, Large Entity. --- End of Maintenance History ---			
Address for fee purposes:	Medarex c/o DARBY & DARBY P.C. P.O. BOX 770 Church Street Station NEW YORK, NY 100080770				
Run Another Query					

Need Help? | USPTO Home Page | Finance Online Shopping Page | Alerts Page

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Customer No 59966 | ISTMT | DATE PRINTED 05/13/2011

Medarex
c/o DARBY & DARBY P.C.
P.O. BOX 770
Church Street Station

MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,984,720	**$980.00**	**$0.00**	**07/10/09**	**09/644,668**	**01/10/06**	**08/24/00**	**04**	**NO**	**MEDRX 0024 (2)**

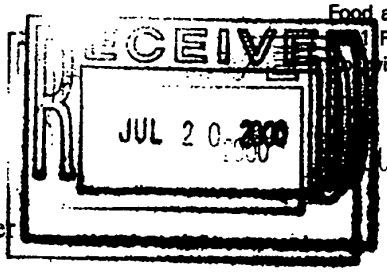
PTOL-439 (Rev. 09/2006)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

6

Food and Drug Administration
[illegible] Rockville Pike
[illegible]ille MD 20852-1448



JUL 18 2000

Our Reference: BB-IND 9186

Medarex, Incorporated
Attention: Randall T. Curnow, M.D.
Senior Vice President and Chief Medical Officer
67 Beaver Avenue
Annandale, NJ 08801

Dear Dr. Curnow:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 9186

SPONSOR: Medarex, Incorporated

PRODUCT NAME: Human Monoclonal Antibody (MDX-CTLA4) to CTLA4

DATE OF SUBMISSION: July 12, 2000

DATE OF RECEIPT: July 13, 2000

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information. All serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, must be reported, in writing, to this Division and to all investigators within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-5101. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research
Attn: Office of Therapeutics Research and Review
HFM-99, Room 200N
1401 Rockville Pike
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Sharon Sickafuse

Sharon Sickafuse. M.S.
Regulatory Project Manager
Division of Application Review and Policy
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Enclosures (3): 21 CFR Part 312
21 CFR 50.20, 50.25
Information sheet on 21 CFR 25.24

for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use for the treatment and/or prevention of nocturnal leg muscle cramps is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After February 22, 1995, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[59 FR 43252, Aug. 22, 1994]

§ 310.547 Drug products containing quinine offered over-the-counter (OTC) for the treatment and/or prevention of malaria.

(a) Quinine and quinine salts have been used OTC for the treatment and/or prevention of malaria, a serious and potentially life-threatening disease. Quinine is no longer the drug of choice for the treatment and/or prevention of most types of malaria. In addition, there are serious and complicating aspects of the disease itself and some potentially serious and life-threatening risks associated with the use of quinine at doses employed for the treatment of malaria. There is a lack of adequate data to establish general recognition of the safety of quinine drug products for OTC use in the treatment and/or prevention of malaria. Therefore, quinine or quinine salts cannot be safely and effectively used for the treatment and/or prevention of malaria except under the care and supervision of a doctor.

(b) Any OTC drug product containing quinine or quinine salts that is labeled, represented, or promoted for the treatment and/or prevention of malaria is regarded as a new drug within the meaning of section 201(p) of the act, for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use for the treatment and/or prevention of malaria is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After April 20, 1998, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[63 FR 13528, Mar. 20, 1998]

PART 312—INVESTIGATIONAL NEW DRUG APPLICATION

Subpart A—General Provisions

Subpart B—Investigational New Drug Application (IND)

Subpart C—Administrative Actions

Subpart D—Responsibilities of Sponsors and Investigators

Subpart E—Drugs Intended to Treat Life-threatening and Severely-debilitating Illnesses

Subpart F—Miscellaneous

Subpart G—Drugs for Investigational Use in Laboratory Research Animals or in Vitro Tests

AUTHORITY: 21 U.S.C. 321, 331, 351, 352, 353, 355, 371; 42 U.S.C. 262.

SOURCE: 52 FR 8831, Mar. 19, 1987, unless otherwise noted.

Subpart A—General Provisions

§ 312.1 Scope.

(a) This part contains procedures and requirements governing the use of investigational new drugs, including procedures and requirements for the submission to, and review by, the Food and Drug Administration of investigational new drug applications (IND's). An investigational new drug for which an IND is in effect in accordance with this part is exempt from the premarketing approval requirements that are otherwise applicable and may be shipped lawfully for the purpose of conducting clinical investigations of that drug.

(b) References in this part to regulations in the Code of Federal Regulations are to chapter I of title 21, unless otherwise noted.

§ 312.2 Applicability.

(a) *Applicability.* Except as provided in this section, this part applies to all clinical investigations of products that are subject to section 505 of the Federal Food, Drug, and Cosmetic Act or to the licensing provisions of the Public Health Service Act (58 Stat. 632, as amended (42 U.S.C. 201 *et seq.*)).

(b) *Exemptions.* (1) The clinical investigation of a drug product that is lawfully marketed in the United States is exempt from the requirements of this part if all the following apply:

(i) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug;

(ii) If the drug that is undergoing investigation is lawfully marketed as a

prescription drug product, the investigation is not intended to support a significant change in the advertising for the product;

(iii) The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;

(iv) The investigation is conducted in compliance with the requirements for institutional review set forth in part 56 and with the requirements for informed consent set forth in part 50; and

(v) The investigation is conducted in compliance with the requirements of § 312.7.

(2)(i) A clinical investigation involving an in vitro diagnostic biological product listed in paragraph (b)(2)(ii) of this section is exempt from the requirements of this part if (*a*) it is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure and (*b*) it is shipped in compliance with § 312.160.

(ii) In accordance with paragraph (b)(2)(i) of this section, the following products are exempt from the requirements of this part: (*a*) blood grouping serum; (*b*) reagent red blood cells; and (*c*) anti-human globulin.

(3) A drug intended solely for tests in vitro or in laboratory research animals is exempt from the requirements of this part if shipped in accordance with § 312.160.

(4) FDA will not accept an application for an investigation that is exempt under the provisions of paragraph (b)(1) of this section.

(5) A clinical investigation involving use of a placebo is exempt from the requirements of this part if the investigation does not otherwise require submission of an IND.

(6) A clinical investigation involving an exception from informed consent under § 50.24 of this chapter is not exempt from the requirements of this part.

(c) *Bioavailability studies.* The applicability of part [illegible] in vivo bioavailability studies in humans is subject to the provisions of § 320.31.

(d) *Unlabeled indication.* This part does not apply to the use in the practice of medicine for an unlabeled indication of a new drug product approved under part 314 or of a licensed biological product.

(e) *Guidance.* FDA may, on its own initiative, issue guidance on the applicability of this part to particular investigational uses of drugs. On request, FDA will advise on the applicability of this part to a planned clinical investigation.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51529, Oct. 2, 1996; 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, § 312.2 was amended by removing "or 507" from paragraph (a) and by removing "or antibiotic drug" from paragraph (d), effective May 20, 1999.

§ 312.3 Definitions and interpretations.

(a) The definitions and interpretations of terms contained in section 201 of the Act apply to those terms when used in this part:

(b) The following definitions of terms also apply to this part:

Act means the Federal Food, Drug, and Cosmetic Act (secs. 201–902, 52 Stat. 1040 *et seq.*, as amended (21 U.S.C. 301–392)).

Clinical investigation means any experiment in which a drug is administered or dispensed to, or used involving, one or more human subjects. For the purposes of this part, an experiment is any use of a drug except for the use of a marketed drug in the course of medical practice.

Contract research organization means a person that assumes, as an independent contractor with the sponsor, one or more of the obligations of a sponsor, e.g., design of a protocol, selection or monitoring of investigations, evaluation of reports, and preparation of materials to be submitted to the Food and Drug Administration.

FDA means the Food and Drug Administration.

IND means an investigational new drug application. For purposes of this part, "IND" is synonymous with "Notice of Claimed Investigational Exemption for a New Drug."

Investigational new drug means a new drug or biological drug that is used in a clinical investigation. The term also includes a biological product that is used in vitro for diagnostic purposes. The terms "investigational drug" and "investigational new drug" are deemed to be synonymous for purposes of this part.

Investigator means an individual who actually conducts a clinical investigation (i.e., under whose immediate direction the drug is administered or dispensed to a subject). In the event an investigation is conducted by a team of individuals, the investigator is the responsible leader of the team. "Subinvestigator" includes any other individual member of that team.

Marketing application means an application for a new drug submitted under section 505(b) of the Act or a product license application for a biological product submitted under the Public Health Service Act.

Sponsor means a person who takes responsibility for and initiates a clinical investigation. The sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization. The sponsor does not actually conduct the investigation unless the sponsor is a sponsor-investigator. A person other than an individual that uses one or more of its own employees to conduct an investigation that it has initiated is a sponsor, not a sponsor-investigator, and the employees are investigators.

Sponsor-Investigator means an individual who both initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. The term does not include any person other than an individual. The requirements applicable to a sponsor-investigator under this part include both those applicable to an investigator and a sponsor.

Subject means a human who participates in an investigation, either as a recipient of the investigational new drug or as a control. A subject may be a healthy human or a patient with a disease.

[52 FR 8831, Mar. 19, 1987, as amended at 64

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, § 312.3 was amended by removing ", antibiotic drug," from the paragraph defining "Investigational new drug" and by removing the phrase ", a request to provide for certification of an antibiotic submitted under section 507 of the Act," from the paragraph defining "Marketing application", effective May 20, 1999.

§ 312.6 Labeling of an investigational new drug.

(a) The immediate package of an investigational new drug intended for human use shall bear a label with the statement "Caution: New Drug—Limited by Federal (or United States) law to investigational use."

(b) The label or labeling of an investigational new drug shall not bear any statement that is false or misleading in any particular and shall not represent that the investigational new drug is safe or effective for the purposes for which it is being investigated.

§ 312.7 Promotion and charging for investigational drugs.

(a) *Promotion of an investigational new drug.* A sponsor or investigator, or any person acting on behalf of a sponsor or investigator, shall not represent in a promotional context that an investigational new drug is safe or effective for the purposes for which it is under investigation or otherwise promote the drug. This provision is not intended to restrict the full exchange of scientific information concerning the drug, including dissemination of scientific findings in scientific or lay media. Rather, its intent is to restrict promotional claims of safety or effectiveness of the drug for a use for which it is under investigation and to preclude commercialization of the drug before it is approved for commercial distribution.

(b) *Commercial distribution of an investigational new drug.* A sponsor or investigator shall not commercially distribute or test market an investigational new drug.

(c) *Prolonging an investigation.* A sponsor shall not unduly prolong an investigation after finding that the results of the investigation appear to establish sufficient data to support a

(d) *Charging for and commercialization of investigational drugs*—(1) *Clinical trials under an IND.* Charging for an investigational drug in a clinical trial under an IND is not permitted without the prior written approval of FDA. In requesting such approval, the sponsor shall provide a full written explanation of why charging is necessary in order for the sponsor to undertake or continue the clinical trial, e.g., why distribution of the drug to test subjects should not be considered part of the normal cost of doing business.

(2) *Treatment protocol or treatment IND.* A sponsor or investigator may charge for an investigational drug for a treatment use under a treatment protocol or treatment IND provided: (i) There is adequate enrollment in the ongoing clinical investigations under the authorized IND; (ii) charging does not constitute commercial marketing of a new drug for which a marketing application has not been approved; (iii) the drug is not being commercially promoted or advertised; and (iv) the sponsor of the drug is actively pursuing marketing approval with due diligence. FDA must be notified in writing in advance of commencing any such charges, in an information amendment submitted under §312.31. Authorization for charging goes into effect automatically 30 days after receipt by FDA of the information amendment, unless the sponsor is notified to the contrary.

(3) *Noncommercialization of investigational drug.* Under this section, the sponsor may not commercialize an investigational drug by charging a price larger than that necessary to recover costs of manufacture, research, development, and handling of the investigational drug.

(4) *Withdrawal of authorization.* Authorization to charge for an investigational drug under this section may be withdrawn by FDA if the agency finds that the conditions underlying the authorization are no longer satisfied.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 19176, May 21, 1987]

§312.10 Waivers.

(a) A sponsor may request FDA to waive applicable requirement under this part. A waiver request may be submitted either in an IND or in an information amendment to an IND. In an emergency, a request may be made by telephone or other rapid communication means. A waiver request is required to contain at least one of the following:

(1) An explanation why the sponsor's compliance with the requirement is unnecessary or cannot be achieved;

(2) A description of an alternative submission or course of action that satisfies the purpose of the requirement; or

(3) Other information justifying a waiver.

(b) FDA may grant a waiver if it finds that the sponsor's noncompliance would not pose a significant and unreasonable risk to human subjects of the investigation and that one of the following is met:

(1) The sponsor's compliance with the requirement is unnecessary for the agency to evaluate the application, or compliance cannot be achieved;

(2) The sponsor's proposed alternative satisfies the requirement; or

(3) The applicant's submission otherwise justifies a waiver.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

Subpart B—Investigational New Drug Application (IND)

§312.20 Requirement for an IND.

(a) A sponsor shall submit an IND to FDA if the sponsor intends to conduct a clinical investigation with an investigational new drug that is subject to §312.2(a).

(b) A sponsor shall not begin a clinical investigation subject to §312.2(a) until the investigation is subject to an IND which is in effect in accordance with §312.40.

(c) A sponsor shall submit a separate IND for any clinical investigation involving an exception from informed consent under §50.24 of this chapter. Such a clinical investigation is not permitted to proceed without the prior written authorization from FDA. FDA shall provide a written determination 30 days after FDA receives the IND or earlier.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51529, Oct. 2, 1996; 62 FR 32179, June 16, 1997]

§312.21 Phases of an investigation.

An IND may be submitted for one or more phases of an investigation. The clinical investigation of a previously untested drug is generally divided into three phases. Although in general the phases are conducted sequentially, they may overlap. These three phases of an investigation are a follows:

(a) *Phase 1.* (1) Phase 1 includes the initial introduction of an investigational new drug into humans. Phase 1 studies are typically closely monitored and may be conducted in patients or normal volunteer subjects. These studies are designed to determine the metabolism and pharmacologic actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on effectiveness. During Phase 1, sufficient information about the drug's pharmacokinetics and pharmacological effects should be obtained to permit the design of well-controlled, scientifically valid, Phase 2 studies. The total number of subjects and patients included in Phase 1 studies varies with the drug, but is generally in the range of 20 to 80.

(2) Phase 1 studies also include studies of drug metabolism, structure-activity relationships, and mechanism of action in humans, as well as studies in which investigational drugs are used as research tools to explore biological phenomena or disease processes.

(b) *Phase 2.* Phase 2 includes the controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks associated with the drug. Phase 2 studies are typically well controlled, closely monitored, and conducted in a relatively small number of patients, usually involving no more than several hundred subjects.

(c) *Phase 3.* Phase 3 studies are expanded controlled and uncontrolled trials. They are performed after preliminary evidence suggesting effectiveness of the drug has been obtained, and are intended to gather the additional information about effectiveness and safety that is needed to evaluate the overall benefit-risk relationship of the drug and to provide an adequate basis for physician labeling. Phase 3 studies usually include from several hundred to several thousand subjects.

§312.22 General principles of the IND submission.

(a) FDA's primary objectives in reviewing an IND are, in all phases of the investigation, to assure the safety and rights of subjects, and, in Phase 2 and 3, to help assure that the quality of the scientific evaluation of drugs is adequate to permit an evaluation of the drug's effectiveness and safety. Therefore, although FDA's review of Phase 1 submissions will focus on assessing the safety of Phase 1 investigations, FDA's review of Phases 2 and 3 submissions will also include an assessment of the scientific quality of the clinical investigations and the likelihood that the investigations will yield data capable of meeting statutory standards for marketing approval.

(b) The amount of information on a particular drug that must be submitted in an IND to assure the accomplishment of the objectives described in paragraph (a) of this section depends upon such factors as the novelty of the drug, the extent to which it has been studied previously, the known or suspected risks, and the developmental phase of the drug.

(c) The central focus of the initial IND submission should be on the general investigational plan and the protocols for specific human studies. Subsequent amendments to the IND that contain new or revised protocols should build logically on previous submissions and should be supported by additional information, including the results of animal toxicology studies or other human studies as appropriate. Annual reports to the IND should use as the focus for reporting the status of studies being conducted under the IND and

should update the general investigational plan for the coming year.

(d) The IND format set forth in §312.23 should be followed routinely by sponsors in the interest of fostering an efficient review of applications. Sponsors are expected to exercise considerable discretion, however, regarding the content of information submitted in each section, depending upon the kind of drug being studied and the nature of the available information. Section 312.23 outlines the information needed for a commercially sponsored IND for a new molecular entity. A sponsor-investigator who uses, as a research tool, an investigational new drug that is already subject to a manufacturer's IND or marketing application should follow the same general format, but ordinarily may, if authorized by the manufacturer, refer to the manufacturer's IND or marketing application in providing the technical information supporting the proposed clinical investigation. A sponsor-investigator who uses an investigational drug not subject to a manufacturer's IND or marketing application is ordinarily required to submit all technical information supporting the IND, unless such information may be referenced from the scientific literature.

§ 312.23 IND content and format.

(a) A sponsor who intends to conduct a clinical investigation subject to this part shall submit an "Investigational New Drug Application" (IND) including, in the following order:

(1) *Cover sheet (Form FDA–1571).* A cover sheet for the application containing the following:

(i) The name, address, and telephone number of the sponsor, the date of the application, and the name of the investigational new drug.

(ii) Identification of the phase or phases of the clinical investigation to be conducted.

(iii) A commitment not to begin clinical investigations until an IND covering the investigations is in effect.

(iv) A commitment that an Institutional Review Board (IRB) that complies with the requirements set forth in part 56 will be responsible for the initial and continuing review and approval of each of the studies in the proposed clinical investigation and the investigator will report to the IRB proposed changes in the research activity in accordance with the requirements of part 56.

(v) A commitment to conduct the investigation in accordance with all other applicable regulatory requirements.

(vi) The name and title of the person responsible for monitoring the conduct and progress of the clinical investigations.

(vii) The name(s) and title(s) of the person(s) responsible under §312.32 for review and evaluation of information relevant to the safety of the drug.

(viii) If a sponsor has transferred any obligations for the conduct of any clinical study to a contract research organization, a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred. If all obligations governing the conduct of the study have been transferred, a general statement of this transfer—in lieu of a listing of the specific obligations transferred—may be submitted.

(ix) The signature of the sponsor or the sponsor's authorized representative. If the person signing the application does not reside or have a place of business within the United States, the IND is required to contain the name and address of, and be countersigned by, an attorney, agent, or other authorized official who resides or maintains a place of business within the United States.

(2) *A table of contents.*

(3) *Introductory statement and general investigational plan.* (i) A brief introductory statement giving the name of the drug and all active ingredients, the drug's pharmacological class, the structural formula of the drug (if known), the formulation of the dosage form(s) to be used, the route of administration, and the broad objectives and planned duration of the proposed clinical investigation(s).

(ii) A brief summary of previous human experience with the drug, with reference to other IND's if pertinent, and to investigational or marketing experience in other countries that may be relevant to the safety of the proposed clinical investigation(s).

(iii) If the drug has been withdrawn from investigation or marketing in any country for any reason related to safety or effectiveness, identification of the country(ies) where the drug was withdrawn and the reasons for the withdrawal.

(iv) A brief description of the overall plan for investigating the drug product for the following year. The plan should include the following: (*a*) The rationale for the drug or the research study; (*b*) the indication(s) to be studied; (*c*) the general approach to be followed in evaluating the drug; (*d*) the kinds of clinical trials to be conducted in the first year following the submission (if plans are not developed for the entire year, the sponsor should so indicate); (*e*) the estimated number of patients to be given the drug in those studies; and (*f*) any risks of particular severity or seriousness anticipated on the basis of the toxicological data in animals or prior studies in humans with the drug or related drugs.

(4) [Reserved]

(5) *Investigator's brochure.* If required under §312.55, a copy of the investigator's brochure, containing the following information:

(i) A brief description of the drug substance and the formulation, including the structural formula, if known.

(ii) A summary of the pharmacological and toxicological effects of the drug in animals and, to the extent known, in humans.

(iii) A summary of the pharmacokinetics and biological disposition of the drug in animals and, if known, in humans.

(iv) A summary of information relating to safety and effectiveness in humans obtained from prior clinical studies. (Reprints of published articles on such studies may be appended when useful.)

(v) A description of possible risks and side effects to be anticipated on the basis of prior experience with the drug under investigation or with related drugs, and of precautions or special monitoring to be done as part of the investigational use of the drug.

(6) *Protocols.* (i) A protocol for each planned study. (Protocols for studies not submitted initially in the IND should be submitted in accordance with §312.30(a).) In general, protocols for Phase 1 studies may be less detailed and more flexible than protocols for Phase 2 and 3 studies. Phase 1 protocols should be directed primarily at providing an outline of the investigation—an estimate of the number of patients to be involved, a description of safety exclusions, and a description of the dosing plan including duration, dose, or method to be used in determining dose—and should specify in detail only those elements of the study that are critical to safety, such as necessary monitoring of vital signs and blood chemistries. Modifications of the experimental design of Phase 1 studies that do not affect critical safety assessments are required to be reported to FDA only in the annual report.

(ii) In Phases 2 and 3, detailed protocols describing all aspects of the study should be submitted. A protocol for a Phase 2 or 3 investigation should be designed in such a way that, if the sponsor anticipates that some deviation from the study design may become necessary as the investigation progresses, alternatives or contingencies to provide for such deviation are built into the protocols at the outset. For example, a protocol for a controlled short-term study might include a plan for an early crossover of nonresponders to an alternative therapy.

(iii) A protocol is required to contain the following, with the specific elements and detail of the protocol reflecting the above distinctions depending on the phase of study:

(*a*) A statement of the objectives and purpose of the study.

(*b*) The name and address and a statement of the qualifications (curriculum vitae or other statement of qualifications) of each investigator, and the name of each subinvestigator (e.g., research fellow, resident) working under the supervision of the investigator; the name and address of the research facilities to be used; and the name and address of each reviewing Institutional Review Board.

(*c*) The criteria for patient selection and for exclusion of patients and an estimate of the number of patients to be studied.

(d) A description of the design of the study, including the kind of control group to be used, if any, and a description of methods to be used to minimize bias on the part of subjects, investigators, and analysts.

(e) The method for determining the dose(s) to be administered, the planned maximum dosage, and the duration of individual patient exposure to the drug.

(f) A description of the observations and measurements to be made to fulfill the objectives of the study.

(g) A description of clinical procedures, laboratory tests, or other measures to be taken to monitor the effects of the drug in human subjects and to minimize risk.

(7) *Chemistry, manufacturing, and control information.* (i) As appropriate for the particular investigations covered by the IND, a section describing the composition, manufacture, and control of the drug substance and the drug product. Although in each phase of the investigation sufficient information is required to be submitted to assure the proper identification, quality, purity, and strength of the investigational drug, the amount of information needed to make that assurance will vary with the phase of the investigation, the proposed duration of the investigation, the dosage form, and the amount of information otherwise available. FDA recognizes that modifications to the method of preparation of the new drug substance and dosage form and changes in the dosage form itself are likely as the investigation progresses. Therefore, the emphasis in an initial Phase 1 submission should generally be placed on the identification and control of the raw materials and the new drug substance. Final specifications for the drug substance and drug product are not expected until the end of the investigational process.

(ii) It should be emphasized that the amount of information to be submitted depends upon the scope of the proposed clinical investigation. For example, although stability data are required in all phases of the IND to demonstrate that the new drug substance and drug product are within acceptable chemical and physical limits for the planned duration of the proposed clinical investigation, if very short-term tests are proposed, the supporting stability data can be correspondingly limited.

(iii) As drug development proceeds and as the scale or production is changed from the pilot-scale production appropriate for the limited initial clinical investigations to the larger-scale production needed for expanded clinical trials, the sponsor should submit information amendments to supplement the initial information submitted on the chemistry, manufacturing, and control processes with information appropriate to the expanded scope of the investigation.

(iv) Reflecting the distinctions described in this paragraph (a)(7), and based on the phase(s) to be studied, the submission is required to contain the following:

(*a*) *Drug substance.* A description of the drug substance, including its physical, chemical, or biological characteristics; the name and address of its manufacturer; the general method of preparation of the drug substance; the acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug substance; and information sufficient to support stability of the drug substance during the toxicological studies and the planned clinical studies. Reference to the current edition of the United States Pharmacopeia—National Formulary may satisfy relevant requirements in this paragraph.

(*b*) *Drug product.* A list of all components, which may include reasonable alternatives for inactive compounds, used in the manufacture of the investigational drug product, including both those components intended to appear in the drug product and those which may not appear but which are used in the manufacturing process, and, where applicable, the quantitative composition of the investigational drug product, including any reasonable variations that may be expected during the investigational stage; the name and address of the drug product manufacturer; a brief general description of the manufacturing and packaging procedures as appropriate for the product; the acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug product; and information sufficient to assure the product's stability during the planned clinical studies. Reference to the current edition of the United States Pharmacopeia—National Formulary may satisfy certain requirements in this paragraph.

(*c*) A brief general description of the composition, manufacture, and control of any placebo used in a controlled clinical trial.

(*d*) *Labeling.* A copy of all labels and labeling to be provided to each investigator.

(*e*) *Environmental analysis requirements.* A claim for categorical exclusion under §25.30 or 25.31 or an environmental assessment under §25.40.

(8) *Pharmacology and toxicology information.* Adequate information about pharmacological and toxicological studies of the drug involving laboratory animals or in vitro, on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and other tests required varies with the duration and nature of the proposed clinical investigations. Guidelines are available from FDA that describe ways in which these requirements may be met. Such information is required to include the identification and qualifications of the individuals who evaluated the results of such studies and concluded that it is reasonably safe to begin the proposed investigations and a statement of where the investigations were conducted and where the records are available for inspection. As drug development proceeds, the sponsor is required to submit informational amendments, as appropriate, with additional information pertinent to safety.

(i) *Pharmacology and drug disposition.* A section describing the pharmacological effects and mechanism(s) of action of the drug in animals, and information on the absorption, distribution, metabolism, and excretion of the drug, if known.

(ii) *Toxicology.* (*a*) An integrated summary of the toxicological effects of the drug in animals and in vitro. Depending on the nature of the drug and the phase of the investigation, the description is to include the results of acute, subacute, and chronic toxicity tests; tests of the drug's effects on reproduction and the developing fetus; any special toxicity test related to the drug's particular mode of administration or conditions of use (e.g., inhalation, dermal, or ocular toxicology); and any in vitro studies intended to evaluate drug toxicity.

(*b*) For each toxicology study that is intended primarily to support the safety of the proposed clinical investigation, a full tabulation of data suitable for detailed review.

(iii) For each nonclinical laboratory study subject to the good laboratory practice regulations under part 58, a statement that the study was conducted in compliance with the good laboratory practice regulations in part 58, or, if the study was not conducted in compliance with those regulations, a brief statement of the reason for the noncompliance.

(9) *Previous human experience with the investigational drug.* A summary of previous human experience known to the applicant, if any, with the investigational drug. The information is required to include the following:

(i) If the investigational drug has been investigated or marketed previously, either in the United States or other countries, detailed information about such experience that is relevant to the safety of the proposed investigation or to the investigation's rationale. If the drug has been the subject of controlled trials, detailed information on such trials that is relevant to an assessment of the drug's effectiveness for the proposed investigational use(s) should also be provided. Any published material that is relevant to the safety of the proposed investigation or to an assessment of the drug's effectiveness for its proposed investigational use should be provided in full. Published material that is less directly relevant may be supplied by a bibliography.

(ii) If the drug is a combination of drugs previously investigated or marketed, the information required under paragraph (a)(9)(i) of this section should be provided for each active drug component. However, if any component in such combination is subject to an approved marketing application or is otherwise lawfully marketed in the

United States, the sponsor is not required to submit published material concerning that active drug component unless such material relates directly to the proposed investigational use (including publications relevant to component-component interaction).

(iii) If the drug has been marketed outside the United States, a list of the countries in which the drug has been marketed and a list of the countries in which the drug has been withdrawn from marketing for reasons potentially related to safety or effectiveness.

(10) *Additional information.* In certain applications, as described below, information on special topics may be needed. Such information shall be submitted in this section as follows:

(i) *Drug dependence and abuse potential.* If the drug is a psychotropic substance or otherwise has abuse potential, a section describing relevant clinical studies and experience and studies in test animals.

(ii) *Radioactive drugs.* If the drug is a radioactive drug, sufficient data from animal or human studies to allow a reasonable calculation of radiation-absorbed dose to the whole body and critical organs upon administration to a human subject. Phase 1 studies of radioactive drugs must include studies which will obtain sufficient data for dosimetry calculations.

(iii) *Pediatric studies.* Plans for assessing pediatric safety and effectiveness.

(iv) *Other information.* A brief statement of any other information that would aid evaluation of the proposed clinical investigations with respect to their safety or their design and potential as controlled clinical trials to support marketing of the drug.

(11) *Relevant information.* If requested by FDA, any other relevant information needed for review of the application.

(b) *Information previously submitted.* The sponsor ordinarily is not required to resubmit information previously submitted, but may incorporate the information by reference. A reference to information submitted previously must identify the file by name, reference number, volume, and page number where the information can be found. A reference to information submitted to the agency by a person other than the sponsor is required to contain a written statement that authorizes the reference and that is signed by the person who submitted the information.

(c) *Material in a foreign language.* The sponsor shall submit an accurate and complete English translation of each part of the IND that is not in English. The sponsor shall also submit a copy of each original literature publication for which an English translation is submitted.

(d) *Number of copies.* The sponsor shall submit an original and two copies of all submissions to the IND file, including the original submission and all amendments and reports.

(e) *Numbering of IND submissions.* Each submission relating to an IND is required to be numbered serially using a single, three-digit serial number. The initial IND is required to be numbered 000; each subsequent submission (e.g., amendment, report, or correspondence) is required to be numbered chronologically in sequence.

(f) *Identification of exception from informed consent.* If the investigation involves an exception from informed consent under §50.24 of this chapter, the sponsor shall prominently identify on the cover sheet that the investigation is subject to the requirements in §50.24 of this chapter.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988; 61 FR 51529, Oct. 2, 1996; 62 FR 10599, July 29, 1997; 63 FR 66669, Dec. 2, 1998]

§ 312.30 Protocol amendments.

Once an IND is in effect, a sponsor shall amend it as needed to ensure that the clinical investigations are conducted according to protocols included in the application. This section sets forth the provisions under which new protocols may be submitted and changes in previously submitted protocols may be made. Whenever a sponsor intends to conduct a clinical investigation with an exception from informed consent for emergency research as set forth in §50.24 of this chapter, the sponsor shall submit a separate IND for such investigation.

(a) *New protocol.* Whenever a sponsor intends to conduct a study that is not covered by a protocol already contained in the IND, the sponsor shall submit to FDA a protocol amendment containing the protocol for the study. Such study may begin provided two conditions are met: (1) The sponsor has submitted the protocol to FDA for its review; and (2) the protocol has been approved by the Institutional Review Board (IRB) with responsibility for review and approval of the study in accordance with the requirements of part 56. The sponsor may comply with these two conditions in either order.

(b) *Changes in a protocol.* (1) A sponsor shall submit a protocol amendment describing any change in a Phase 1 protocol that significantly affects the safety of subjects or any change in a Phase 2 or 3 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of changes requiring an amendment under this paragraph include:

(i) Any increase in drug dosage or duration of exposure of individual subjects to the drug beyond that in the current protocol, or any significant increase in the number of subjects under study.

(ii) Any significant change in the design of a protocol (such as the addition or dropping of a control group).

(iii) The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or adverse event; or the dropping of a test intended to monitor safety.

(2)(i) A protocol change under paragraph (b)(1) of this section may be made provided two conditions are met:

(a) The sponsor has submitted the change to FDA for its review; and

(b) The change has been approved by the IRB with responsibility for review and approval of the study. The sponsor may comply with these two conditions in either order.

(ii) Notwithstanding paragraph (b)(2)(i) of this section, a protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately provided FDA is subsequently notified by protocol amendment and the reviewing IRB is notified in accordance with §56.104(c).

(c) *New investigator.* A sponsor shall submit a protocol amendment when a new investigator is added to carry out a previously submitted protocol, except that a protocol amendment is not required when a licensed practitioner is added in the case of a treatment protocol under §312.34. Once the investigator is added to the study, the investigational drug may be shipped to the investigator and the investigator may begin participating in the study. The sponsor shall notify FDA of the new investigator within 30 days of the investigator being added.

(d) *Content and format.* A protocol amendment is required to be prominently identified as such (i.e., "Protocol Amendment: New Protocol", "Protocol Amendment: Change in Protocol", or "Protocol Amendment: New Investigator"), and to contain the following:

(1)(i) In the case of a new protocol, a copy of the new protocol and a brief description of the most clinically significant differences between it and previous protocols.

(ii) In the case of a change in protocol, a brief description of the change and reference (date and number) to the submission that contained the protocol.

(iii) In the case of a new investigator, the investigator's name, the qualifications to conduct the investigation, reference to the previously submitted protocol, and all additional information about the investigator's study as is required under §312.23(a)(6)(iii)(*b*).

(2) Reference, if necessary, to specific technical information in the IND or in a concurrently submitted information amendment to the IND that the sponsor relies on to support any clinically significant change in the new or amended protocol. If the reference is made to supporting information already in the IND, the sponsor shall identify by name, reference number, volume, and page number the location of the information.

(3) If the sponsor desires FDA to comment on the submission, a request for such comment and the specific questions FDA's response should address.

(c) *When submitted.* A sponsor shall submit a protocol amendment for a new protocol or a change in protocol before its implementation. Protocol amendments to add a new investigator or to provide additional information about investigators may be grouped and submitted at 30-day intervals. When several submissions of new protocols or protocol changes are anticipated during a short period, the sponsor is encouraged, to the extent feasible, to include these all in a single submission.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988; 61 FR 51530, Oct. 2, 1996]

§312.31 Information amendments.

(a) *Requirement for information amendment.* A sponsor shall report in an information amendment essential information on the IND that is not within the scope of a protocol amendment, IND safety reports, or annual report. Examples of information requiring an information amendment include:

(1) New toxicology, chemistry, or other technical information; or

(2) A report regarding the discontinuance of a clinical investigation.

(b) *Content and format of an information amendment.* An information amendment is required to bear prominent identification of its contents (e.g., "Information Amendment: Chemistry, Manufacturing, and Control", "Information Amendment: Pharmacology-Toxicology", "Information Amendment: Clinical"), and to contain the following:

(1) A statement of the nature and purpose of the amendment.

(2) An organized submission of the data in a format appropriate for scientific review.

(3) If the sponsor desires FDA to comment on an information amendment, a request for such comment.

(c) *When submitted.* Information amendments to the IND should be submitted as necessary but, to the extent feasible, not more than every 30 days.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988]

§312.32 IND safety reports.

(a) *Definitions.* The following definitions of terms apply to this section:

Associated with the use of the drug. There is a reasonable possibility that the experience may have been caused by the drug.

Disability. A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse drug experience. Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse drug experience: Any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected adverse drug experience: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed cerebral vascular accidents. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

(b) *Review of safety information.* The sponsor shall promptly review all information relevant to the safety of the drug obtained or otherwise received by the sponsor from any source, foreign or domestic, including information derived from any clinical or epidemiological investigations, animal investigations, commercial marketing experience, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities that have not already been previously reported to the agency by the sponsor.

(c) *IND safety reports.* (1) *Written reports.*—(i) The sponsor shall notify FDA and all participating investigators in a written IND safety report of:

(A) Any adverse experience associated with the use of the drug that is both serious and unexpected; or

(B) Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification shall be made as soon as possible and in no event later than 15 calendar days after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A or in a narrative format (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form; reports from animal or epidemiological studies shall be submitted in a narrative format) and shall bear prominent identification of its contents, i.e., "IND Safety Report." Each written notification to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND. If FDA determines that additional data are needed, the agency may require further data to be submitted.

(ii) In each written IND safety report, the sponsor shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previouos, similar reports.

(2) *Telephone and facsimile transmission safety reports.* The sponsor shall also notify FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but in no event later than 7 calendar days after the sponsor's initial receipt of the information. Each telephone call or facsimile transmission to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND.

(3) *Reporting format or frequency.* FDA may request a sponsor to submit IND safety reports in a format or at a frequency different than that required under this paragraph. The sponsor may also propose and adopt a different reporting format or frequency if the change is agreed to in advance by the director of the new drug review division in the Center for Drug Evaluation and Research or the director of the products review division in the Center for Biologics Evaluation and Research which is responsible for review of the IND.

(4) A sponsor of a clinical study of a marketed drug is not required [illegible]

a safety report for any adverse experience associated with use of the drug that is not from the clinical study itself.

(d) *Followup.* (1) The sponsor shall promptly investigate all safety information received by it.

(2) Followup information to a safety report shall be submitted as soon as the relevant information is available.

(3) If the results of a sponsor's investigation show that an adverse drug experience not initially determined to be reportable under paragraph (c) of this section is so reportable, the sponsor shall report such experience in a written safety report as soon as possible, but in no event later than 15 calendar days after the determination is made.

(4) Results of a sponsor's investigation of other safety information shall be submitted, as appropriate, in an information amendment or annual report.

(e) *Disclaimer.* A safety report or other information submitted by a sponsor under this part (and any release by FDA of that report or information) does not necessarily reflect a conclusion by the sponsor or FDA that the report or information constitutes an admission that the drug caused or contributed to an adverse experience. A sponsor need not admit, and may deny, that the report or information submitted by the sponsor constitutes an admission that the drug caused or contributed to an adverse experience.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11679, Mar. 29, 1990; 62 FR 52250, Oct. 7, 1997]

§ 312.33 Annual reports.

A sponsor shall within 60 days of the anniversary date that the IND went into effect, submit a brief report of the progress of the investigation that includes:

(a) *Individual study information.* A brief summary of the status of each study in progress and each study completed during the previous year. The summary is required to include the following information for each study:

(1) The title of the study (with any appropriate . . y identifiers such as protocol number), its purpose, a brief statement identifying the patient population, and a statement as to whether the study is completed.

(2) The total number of subjects initially planned for inclusion in the study; the number entered into the study to date, tabulated by age group, gender, and race; the number whose participation in the study was completed as planned; and the number who dropped out of the study for any reason.

(3) If the study has been completed, or if interim results are known, a brief description of any available study results.

(b) *Summary information.* Information obtained during the previous year's clinical and nonclinical investigations, including:

(1) A narrative or tabular summary showing the most frequent and most serious adverse experiences by body system.

(2) A summary of all IND safety reports submitted during the past year.

(3) A list of subjects who died during participation in the investigation, with the cause of death for each subject.

(4) A list of subjects who dropped out during the course of the investigation in association with any adverse experience, whether or not thought to be drug related.

(5) A brief description of what, if anything, was obtained that is pertinent to an understanding of the drug's actions, including, for example, information about dose response, information from controlled trails, and information about bioavailability.

(6) A list of the preclinical studies (including animal studies) completed or in progress during the past year and a summary of the major preclinical findings.

(7) A summary of any significant manufacturing or microbiological changes made during the past year.

(c) A description of the general investigational plan for the coming year to replace that submitted 1 year earlier. The general investigational plan shall contain the information required under §312.23(a)(3)(iv).

(d) If the investigator brochure has been revised, a description of the revision and a copy of the new brochure.

(e) A description of any significant Phase 1 protocol modifications made during the previous year and not previously reported to the IND in a protocol amendment.

(f) A brief summary of significant foreign marketing developments with the drug during the past year, such as approval of marketing in any country or withdrawal or suspension from marketing in any country.

(g) If desired by the sponsor, a log of any outstanding business with respect to the IND for which the sponsor requests or expects a reply, comment, or meeting.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 63 FR 6862, Feb. 11, 1998]

§ 312.34 Treatment use of an investigational new drug.

(a) *General.* A drug that is not approved for marketing may be under clinical investigation for a serious or immediately life-threatening disease condition in patients for whom no comparable or satisfactory alternative drug or other therapy is available. During the clinical investigation of the drug, it may be appropriate to use the drug in the treatment of patients not in the clinical trials, in accordance with a treatment protocol or treatment IND. The purpose of this section is to facilitate the availability of promising new drugs to desperately ill patients as early in the drug development process as possible, before general marketing begins, and to obtain additional data on the drug's safety and effectiveness. In the case of a serious disease, a drug ordinarily may be made available for treatment use under this section during Phase 3 investigations or after all clinical trials have been completed; however, in appropriate circumstances, a drug may be made available for treatment use during Phase 2. In the case of an immediately life-threatening disease, a drug may be made available for treatment use under this section earlier than Phase 3, but ordinarily not earlier than Phase 2. For purposes of this section, the "treatment use" of a drug includes the use of a drug for diagnostic purposes. If a protocol for an investigational drug meets the criteria of this section, the protocol is to be submitted as a treatment protocol under the provisions of this section.

(b) *Criteria.* (1) FDA shall permit an investigational drug to be used for a treatment use under a treatment protocol or treatment IND if:

(i) The drug is intended to treat a serious or immediately life-threatening disease;

(ii) There is no comparable or satisfactory alternative drug or other therapy available to treat that stage of the disease in the intended patient population;

(iii) The drug is under investigation in a controlled clinical trial under an IND in effect for the trial, or all clinical trials have been completed; and

(iv) The sponsor of the controlled clinical trial is actively pursuing marketing approval of the investigational drug with due diligence.

(2) *Serious disease.* For a drug intended to treat a serious disease, the Commissioner may deny a request for treatment use under a treatment protocol or treatment IND if there is insufficient evidence of safety and effectiveness to support such use.

(3) *Immediately life-threatening disease.* (i) For a drug intended to treat an immediately life-threatening disease, the Commissioner may deny a request for treatment use of an investigational drug under a treatment protocol or treatment IND if the available scientific evidence, taken as a whole, fails to provide a reasonable basis for concluding that the drug:

(A) May be effective for its intended use in its intended patient population; or

(B) Would not expose the patients to whom the drug is to be administered to an unreasonable and significant additional risk of illness or injury.

(ii) For the purpose of this section, an "immediately life-threatening" disease means a stage of a disease in which there is a reasonable likelihood that death will occur within a matter of months or in which premature death is likely without early . . tment.

(c) *Safeguards.* Treatment use of an investigational drug is conditioned on

he sponsor and investigators complying with the safeguards of the IND rocess, including the regulations governing informed consent (21 CFR part 0) and institutional review boards (21 FR part 56) and the applicable provisions of part 312, including distribution f the drug through qualified experts, aintenance of adequate manufacuring facilities, and submission of IND afety reports.

(d) *Clinical hold.* FDA may place on linical hold a proposed or ongoing eatment protocol or treatment IND accordance with §312.42.

2 FR 19476, May 22, 1987, as amended at 57 R 13248, Apr. 15, 1992]

312.35 Submissions for treatment use.

(a) *Treatment protocol submitted by VD sponsor.* Any sponsor of a clinical ivestigation of a drug who intends to onsor a treatment use for the drug all submit to FDA a treatment proocol under §312.34 if the sponsor beeves the criteria of §312.34 are satised. If a protocol is not submitted nder §312.34, but FDA believes that ie protocol should have been subitted under this section, FDA may em the protocol to be submitted der §312.34. A treatment use under a eatment protocol may begin 30 days ter FDA receives the protocol or on rlier notification by FDA that the eatment use described in the protocol ay begin.

(1) A treatment protocol is required contain the following:

(i) The intended use of the drug.

(ii) An explanation of the rationale r use of the drug, including, as appropiate, either a list of what available gimens ordinarily should be tried bere using the investigational drug or explanation of why the use of the vestigational drug is preferable to e use of available marketed treatents.

(iii) A brief description of the criteria r patient selection.

(iv) The method of administration of e drug and the dosages.

(v) A description of clinical procees, laboratory te or other meases to monitor the ects of the drug d to minimize risk.

(2) A treatment protocol is to be supported by the following:

(i) Informational brochure for supplying to each treating physician.

(ii) The technical information that is relevant to safety and effectiveness of the drug for the intended treatment purpose. Information contained in the sponsor's IND may be incorporated by reference.

(iii) A commitment by the sponsor to assure compliance of all participating investigators with the informed consent requirements of 21 CFR part 50.

(3) A licensed practioner who receives an investigational drug for treatment use under a treatment protocol is an "investigator" under the protocol and is responsible for meeting all applicable investigator responsibilities under this part and 21 CFR parts 50 and 56.

(b) *Treatment IND submitted by licensed practitioner.* (1) If a licensed medical practitioner wants to obtain an investigational drug subject to a controlled clinical trial for a treatment use, the practitioner should first attempt to obtain the drug from the sponsor of the controlled trial under a treatment protocol. If the sponsor of the controlled clinical investigation of the dru will not establish a treatment protocol for the drug under paragraph (a) of this section, the licensed medical practitioner may seek to obtain the drug from the sponsor and submit a treatment IND to FDA requesting authorization to use the investigational drug for treatment use. A treatment use under a treatment IND may begin 30 days after FDA receives the IND or on earlier notification by FDA that the treatment use under the IND may begin. A treatment IND is required to contain the following:

(i) A cover sheet (Form FDA 1571) meeting §312.23(g)(1).

(ii) Information (when not provided by the sponsor) on the drug's chemistry, manufacturing, a ontrols, and prior clinical and nonclinical experience with the drug submitted in accordance with §312.23. A sponsor of a clinical investigation subject to an IND who supplies an investigational drug to a licensed medical practitioner for purposes of a separate treatment clinical investigation shall be deemed to authorize the incorporation-by-reference of the technical information contained in the sponsor's IND into the medical practitioner's treatment IND.

(iii) A statement of the steps taken by the practitioner to obtain the drug under a treatment protocol from the drug sponsor.

(iv) A treatment protocol containing the same information listed in paragraph (a)(1) of this section.

(v) A statement of the practitioner's qualifications to use the investigational drug for the intended treatment use.

(vi) The practitioner's statement of familiarity with information on the drug's safety and effectiveness derived from previous clinical and nonclinical experience with the drug.

(vii) Agreement to report to FDA safety information in accordance with §312.32.

(2) A licensed practitioner who submits a treatment IND under this section is the sponsor-investigator for such IND and is responsible for meeting all applicable sponsor and investigator responsibilities under this part and 21 CFR parts 50 and 56.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 19477, May 22, 1987, as amended at 57 FR 13219, Apr. 15, 1992]

§312.36 Emergency use of an investigational new drug.

Need for an investigational drug may arise in an emergency situation that does not allow time for submission of an IND in accordance with §312.23 or §312.34. In such a case, FDA may authorize shipment of the drug for a specified use in advance of submission of an IND. A request for such authorization may be transmitted to FDA by telephone or other rapid communication means. For investigational biological drugs, the request should be directed to the Division of Biological Investigational New Drugs (HFB-230), Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, MD 20892, 301-443-4864. For all other investigational drugs, the request for authorization should be directed to the ument Management and Reporting anch (HFD-53), Center for Drug Evaluation and Research, 5600 Fishers Lane, Rockville, MD 20857, 301-443-4320. After normal working hours, eastern standard time, the request should be directed to the FDA Division of Emergency and Epidemiological Operations, 202-857-8400. Except in extraordinary circumstances, such authorization will be conditioned on the sponsor making an appropriate IND submission as soon as practicable after receiving the authorization.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990]

§312.38 Withdrawal of an IND.

(a) At any time a sponsor may withdraw an effective IND without prejudice.

(b) If an IND is withdrawn, FDA shall be so notified, all clinical investigations conducted under the IND shall be ended, all current investigators notified, and all stocks of the drug returned to the sponsor or otherwise disposed of at the request of the sponsor in accordance with §312.59.

(c) If an IND is withdrawn because of a safety reason, the sponsor shall promptly so inform FDA, all participating investigators, and all reviewing Institutional Review Boards, together with the reasons for such withdrawal.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

Subpart C—Administrative Actions

§312.40 General requirements for use of an investigational new drug in a clinical investigation.

(a) An investigational new drug may be used in a clinical investigation if the following conditions are met:

(1) The sponsor of the investigation submits an IND for the drug to FDA; the IND is in effect under paragraph (b) of this section; and the sponsor complies with all applicable re ements in this part and parts 50 and with respect to the conduct of the clinical in-

(2) Each participating investigator conducts his or her investigation in compliance with the requirements of this part and parts 50 and 56.

(b) An IND goes into effect:

(1) Thirty days after FDA receives the IND, unless FDA notifies the sponsor that the investigations described in the IND are subject to a clinical hold under §312.42; or

(2) On earlier notification by FDA that the clinical investigations in the IND may begin. FDA will notify the sponsor in writing of the date it receives the IND.

(c) A sponsor may ship an investigational new drug to investigators named in the IND:

(1) Thirty days after FDA receives the IND; or

(2) On earlier FDA authorization to ship the drug.

(d) An investigator may not administer an investigational new drug to human subjects until the IND goes into effect under paragraph (b) of this section.

§312.41 Comment and advice on an IND.

(a) FDA may at any time during the course of the investigation communicate with the sponsor orally or in writing about deficiencies in the IND or about FDA's need for more data or information.

(b) On the sponsor's request, FDA will provide advice on specific matters relating to an IND. Examples of such advice may include advice on the adequacy of technical data to support an investigational plan, on the design of a clinical trial, and on whether proposed investigations are likely to produce the data and information that is needed to meet requirements for a marketing application.

(c) Unless the communication is accompanied by a clinical hold order under §312.42, FDA communications with a sponsor under this section are solely advisory and do not require any modification in the planned or ongoing clinical investigations or response to the agency.

[Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014]

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§312.42 Clinical holds and requests for modification.

(a) *General.* A clinical hold is an order issued by FDA to the sponsor to delay a proposed clinical investigation or to suspend an ongoing investigation. The clinical hold order may apply to one or more of the investigations covered by an IND. When a proposed study is placed on clinical hold, subjects may not be given the investigational drug. When an ongoing study is placed on clinical hold, no new subjects may be recruited to the study and placed on the investigational drug; patients already in the study should be taken off therapy involving the investigational drug unless specifically permitted by FDA in the interest of patient safety.

(b) *Grounds for imposition of clinical hold—(1) Clinical hold of a Phase 1 study under an IND.* FDA may place a proposed or ongoing Phase 1 investigation on clinical hold if it finds that:

(i) Human subjects are or would be exposed to an unreasonable and significant risk of illness or injury;

(ii) The clinical investigators named in the IND are not qualified by reason of their scientific training and experience to conduct the investigation described in the IND;

(iii) The investigator brochure is misleading, erroneous, or materially incomplete; or

(iv) The IND does not contain sufficient information required under §312.23 to assess the risks to subjects of the proposed studies.

(2) *Clinical hold of a Phase 2 or 3 study under an IND.* FDA may place a proposed or ongoing Phase 2 or 3 investigation on clinical hold if it finds that:

(i) Any of the conditions in paragraph (b)(1)(i) through (iv) of this section apply; or

(ii) The plan or protocol for the investigation is clearly deficient in design to meet its stated objectives.

(3) Clinical hold of a treatment IND or treatment protocol.

(i) *Proposed use.* FDA may place a proposed treatment IND or treatment protocol on clinical hold if it is determined that:

(A) The pertinent criteria in §312.34(b) for permitting the treatment use to begin are not satisfied; or

(B) The treatment protocol or treatment IND does not contain the information required under §312.35 (a) or (b) to make the specified determination under §312.31(b).

(ii) *Ongoing use.* FDA may place an ongoing treatment protocol or treatment IND on clinical hold if it is determined that:

(A) There becomes available a comparable or satisfactory alternative drug or other therapy to treat that stage of the disease in the intended patient population for which the investigational drug is being used;

(B) The investigational drug is not under investigation in a controlled clinical trial under an IND in effect for the trial and not all controlled clinical trials necessary to support a marketing application have been completed, or a clinical study under the IND has been placed on clinical hold;

(C) The sponsor of the controlled clinical trial is not pursuing marketing approval with due diligence;

(D) If the treatment IND or treatment protocol is intended for a serious disease, there is insufficient evidence of safety and effectiveness to support such use; or

(E) If the treatment protocol or treatment IND was based on an immediately life-threatening disease, the available scientific evidence, taken as a whole, fails to provide a reasonable basis for concluding that the drug:

(*1*) May be effective for its intended use in its intended population; or

(*2*) Would not expose the patients to whom the drug is to be administered to an unreasonable and significant additional risk of illness or injury.

(iii) FDA may place a proposed or ongoing treatment IND or treatment protocol on clinical hold if it finds that any of the conditions in paragraph (b)(4)(i) through (b)(4)(viii) of this section apply.

(4) *Clinical hold of any study that is not designed to be adequate and well-controlled.* FDA may place a proposed or ongoing investigation that is not designed to be adequate and well-controlled on clinical hold if it finds that:

(i) Any of the conditions in paragraph (b)(1) or (b)(2) of this section apply; or

(ii) There is reasonable evidence the investigation that is not designed to be adequate and well-controlled is impeding enrollment in, or otherwise interfering with the conduct or completion of, a study that is designed to be an adequate and well-controlled investigation of the same or another investigational drug; or

(iii) Insufficient quantities of the investigational drug exist to adequately conduct both the investigation that is not designed to be adequate and well-controlled and the investigations that are designed to be adequate and well-controlled; or

(iv) The drug has been studied in one or more adequate and well-controlled investigations that strongly suggest lack of effectiveness; or

(v) Another drug under investigation or approved for the same indication and available to the same patient population has demonstrated a better potential benefit/risk balance; or

(vi) The drug has received marketing approval for the same indication in the same patient population; or

(vii) The sponsor of the study that is designed to be an adequate and well-controlled investigation is not actively pursuing marketing approval of the investigational drug with due diligence; or

(viii) The Commissioner determines that it would not be in the public interest for the study to be conducted or continued. FDA ordinarily intends that clinical holds under paragraphs (b)(4)(ii), (b)(4)(iii) and (b)(4)(v) of this section would only apply to additional enrollment in nonconcurrently controlled trials rather than eliminating continued access to individuals already receiving the investigational drug.

(5) *Clinical hold of any investigation involving an exception from informed consent under* [illegible]

may place a proposed or ongoing investigation involving an exception from informed consent under §50.24 of this chapter on clinical hold if it is determined that:

(i) Any of the conditions in paragraphs (b)(1) or (b)(2) of this section apply; or

(ii) The pertinent criteria in §50.24 of this chapter for such an investigation to begin or continue are not submitted or not satisfied.

(c) *Discussion of deficiency.* Whenever FDA concludes that a deficiency exists in a clinical investigation that may be grounds for the imposition of clinical hold FDA will, unless patients are exposed to immediate and serious risk, attempt to discuss and satisfactorily resolve the matter with the sponsor before issuing the clinical hold order.

(d) *Imposition of clinical hold.* The clinical hold order may be made by telephone or other means of rapid communication or in writing. The clinical hold order will identify the studies under the IND to which the hold applies, and will briefly explain the basis for the action. The clinical hold order will be made by or on behalf of the Division Director with responsibility for review of the IND. As soon as possible, and no more than 30 days after imposition of the clinical hold, the Division Director will provide the sponsor a written explanation of the basis for the hold.

(e) *Resumption of clinical investigations.* An investigation may only resume after FDA (usually the Division Director, or the Director's designee, with responsibility for review of the IND) has notified the sponsor that the investigation may proceed. Resumption of the affected investigation(s) will be authorized when the sponsor corrects the deficiency(ies) previously cited or otherwise satisfies the agency that the investigation(s) can proceed. FDA may notify a sponsor of its determination regarding the clinical hold by telephone or other means of rapid communication. If a sponsor of an IND that has been placed on clinical hold requests in writing that the clinical hold be removed and submits a complete response to the issue(s) identified in the clinical hold order, FDA shall respond in writing to the sponsor within 30-calendar days of receipt of the request as the complete response. FDA's response will either remove or maintain the clinical hold, and will state the reasons for such determination. Notwithstanding the 30-calendar day response time, a sponsor may not proceed with a clinical trial on which a clinical hold has been imposed until the sponsor has been notified by FDA that the hold has been lifted.

(f) *Appeal.* If the sponsor disagrees with the reasons cited for the clinical hold, the sponsor may request reconsideration of the decision in accordance with §312.48.

(g) *Conversion of IND on clinical hold to inactive status.* If all investigations covered by an IND remain on clinical hold for 1 year or more, the IND may be placed on inactive status by FDA under §312.45.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 19477, May 22, 1987; 57 FR 13249, Apr. 15, 1992; 61 FR 51530, Oct. 2, 1996; 63 FR 68678, Dec. 14, 1998]

EFFECTIVE DATE NOTE: At 63 FR 68678, Dec. 14, 1998, §312.42 was amended by revising paragraph (e), effective Apr. 28, 1999. For the convenience of the user, the superseded text follows:

§312.42 Clinical holds and requests for modification.

* * * * *

(e) *Resumption of clinical investigations.* If, by the terms of the clinical hold order, resumption of the affected investigation is permitted without prior notification by FDA once a stated correction or modification is made, the investigation may proceed as soon as the correction or modification is made. In all other cases, an investigation may only resume after the Division Director (or the Director's designee) with responsibility for review of the IND has notified the sponsor that the investigation may proceed. In these cases resumption of the affected investigation(s) will be authorized when the sponsor corrects the deficiency(ies) previously cited or otherwise satisfied the agency that the investigation(s) can proceed. Resumption of a study may be authorized by telephone or other means of rapid communication.

* * * * *

§312.44 Termination.

(a) *General.* This section describes the procedures under which FDA may terminate an IND. If an IND is terminated, the sponsor shall end all clinical investigations conducted under the IND and recall or otherwise provide for the disposition of all unused supplies of the drug. A termination action may be based on deficiencies in the IND or in the conduct of an investigation under an IND. Except as provided in paragraph (d) of this section, a termination shall be preceded by a proposal to terminate by FDA and an opportunity for the sponsor to respond. FDA will, in general, only initiate an action under this section after first attempting to resolve differences informally or, when appropriate, through the clinical hold procedures described in §312.42.

(b) *Grounds for termination—(1) Phase 1.* FDA may propose to terminate an IND during Phase 1 if it finds that:

(i) Human subjects would be exposed to an unreasonable and significant risk of illness or injury.

(ii) The IND does not contain sufficient information required under §312.23 to assess the safety to subjects of the clinical investigations.

(iii) The methods, facilities, and controls used for the manufacturing, processing, and packing of the investigational drug are inadequate to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for subject safety.

(iv) The clinical investigations are being conducted in a manner substantially different than that described in the protocols submitted in the IND.

(v) The drug is being promoted or distributed for commercial purposes not justified by the requirements of the investigation or permitted by §312.7.

(vi) The IND, or any amendment or report to the IND, contains an untrue statement of a material fact or omits material information required by this part.

(vii) The sponsor fails promptly to investigate and inform the Food and Drug Administration and all investigators of serious and unexpected adverse experiences in accordance with §312.32 or fails to make any other report required under this part.

(viii) The sponsor fails to submit an accurate annual report of the investigations in accordance with §312.33.

(ix) The sponsor fails to comply with any other applicable requirement of this part, part 50, or part 56.

(x) The IND has remained on inactive status for 5 years or more.

(xi) The sponsor fails to delay a proposed investigation under the IND or to suspend an ongoing investigation that has been placed on clinical hold under §312.42(b)(4).

(2) *Phase 2 or 3.* FDA may propose to terminate an IND during Phase 2 or Phase 3 if FDA finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (b)(1)(xi) of this section apply; or

(ii) The investigational plan or protocol(s) is not reasonable as a bona fide scientific plan to determine whether or not the drug is safe and effective for use; or

(iii) There is convincing evidence that the drug is not effective for the purpose for which it is being investigated.

(3) FDA may propose to terminate a treatment IND if it finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (x) of this section apply; or

(ii) Any of the conditions in §312.42(b)(3) apply.

(c) *Opportunity for sponsor response.* (1) If FDA proposes to terminate an IND, FDA will notify the sponsor in writing, and invite correction or explanation within a period of 30 days.

(2) On such notification, the sponsor may provide a written explanation or correction or may request a conference with FDA to provide the requested explanation or correction. If the sponsor does not respond to the notification within the allocated time, the IND shall be terminated.

(3) If the sponsor responds but FDA does not accept the explanation or correction submitted, FDA shall inform the sponsor in writing of the reason for the nonacceptance and provide the sponsor with an opportunity for a regulatory hearing before FDA under part 16 on the question of whether the IND should be terminated. The sponsor's request for a regulatory hearing must be made within 10 days of the

receipt of FDA's notification of nonacceptance.

(d) *Immediate termination of IND.* Notwithstanding paragraphs (a) through (c) of this section, if at any time FDA concludes that continuation of the investigation presents an immediate and substantial danger to the health of individuals, the agency shall immediately, by written notice to the sponsor from the Director of the Center for Drug Evaluation and Research or the Director of the Center for Biologics Evaluation and Research, terminate the IND. An IND so terminated is subject to reinstatement by the Director on the basis of additional submissions that eliminate such danger. If an IND is terminated under this paragraph, the agency will afford the sponsor an opportunity for a regulatory hearing under part 16 on the question of whether the IND should be reinstated.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990; 57 FR 13249, Apr. 15, 1992]

§ 312.45 Inactive status.

(a) If no subjects are entered into clinical studies for a period of 2 years or more under an IND, or if all investigations under an IND remain on clinical hold for 1 year or more, the IND may be placed by FDA on inactive status. This action may be taken by FDA either on request of the sponsor or on FDA's own initiative. If FDA seeks to act on its own initiative under this section, it shall first notify the sponsor in writing of the proposed inactive status. Upon receipt of such notification, the sponsor shall have 30 days to respond as to why the IND should continue to remain active.

(b) If an IND is placed on inactive status, all investigators shall be so notified and all stocks of the drug shall be returned or otherwise disposed of in accordance with § 312.59.

(c) A sponsor is not required to submit annual reports to an IND on inactive status. An inactive IND is, however, still in effect for purposes of the public disclosure data and information under § 312.130.

(d) A sponsor who intends to resume clinical investigation under an IND placed on inactive status shall submit a protocol amendment under § 312.30 containing the proposed general investigational plan for the coming year and appropriate protocols. If the protocol amendment relies on information previously submitted, the plan shall reference such information. Additional information supporting the proposed investigation, if any, shall be submitted in an information amendment. Notwithstanding the provisions of § 312.30, clinical investigations under an IND on inactive status may only resume (1) 30 days after FDA receives the protocol amendment, unless FDA notifies the sponsor that the investigations described in the amendment are subject to a clinical hold under § 312.42, or (2) on earlier notification by FDA that the clinical investigations described in the protocol amendment may begin.

(e) An IND that remains on inactive status for 5 years or more may be terminated under § 312.44.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.47 Meetings.

(a) *General.* Meetings between a sponsor and the agency are frequently useful in resolving questions and issues raised during the course of a clinical investigation. FDA encourages such meetings to the extent that they aid in the evaluation of the drug and in the solution of scientific problems concerning the drug, to the extent that FDA's resources permit. The general principle underlying the conduct of such meetings is that there should be free, full, and open communication about any scientific or medical question that may arise during the clinical investigation. These meetings shall be conducted and documented in accordance with part 10.

(b) *"End-of-Phase 2" meetings and meetings held before submission of a marketing application.* At specific times during the drug investigation process, meetings between FDA and a sponsor can be especially helpful in minimizing wasteful expenditures of time and money and thus in speeding the drug development and evaluation process. In particular, FDA has found that meetings at the end of Phase 2 of an investigation (end-of-Phase 2 meetings) are of considerable assistance in planning later studies and that meetings held near completion of Phase 3 and before submission of a marketing application ("pre-NDA" meetings) are helpful in developing methods of presentation and submission of data in the marketing application that facilitate review and allow timely FDA response.

(1) *End-of-Phase 2 meetings*—(i) *Purpose.* The purpose of an end-of-phase 2 meeting is to determine the safety of proceeding to Phase 3, to evaluate the Phase 3 plan and protocols and the adequacy of current studies and plans to assess pediatric safety and effectiveness, and to identify any additional information necessary to support a marketing application for the uses under investigation.

(ii) *Eligibility for meeting.* While the end-of-Phase 2 meeting is designed primarily for IND's involving new molecular entities or major new uses of marketed drugs, a sponsor of any IND may request and obtain an end-of-Phase 2 meeting.

(iii) *Timing.* To be most useful to the sponsor, end-of-Phase 2 meetings should be held before major commitments of effort and resources to specific Phase 3 tests are made. The scheduling of an end-of-Phase 2 meeting is not, however, intended to delay the transition of an investigation from Phase 2 to Phase 3.

(iv) *Advance information.* At least 1 month in advance of an end-of-Phase 2 meeting, the sponsor should submit background information on the sponsor's plan for Phase 3, including summaries of the Phase 1 and 2 investigations, the specific protocols for Phase 3 clinical studies, plans for any additional nonclinical studies, plans for pediatric studies, including a time line for protocol finalization, enrollment, completion, and data analysis, or information to support any planned request for waiver or deferral of pediatric studies and, if available, tentative labeling for the drug. The recommended contents of such a submission are described more fully in FDA Staff Manual Guide 4850.7 that is publicly available under FDA's public information regulations in part 20.

(v) *Conduct of meeting.* Arrangements for an end-of-Phase 2 meeting are to be made with the division in FDA's Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research which is responsible for review of the IND. The meeting will be scheduled by FDA at a time convenient to both FDA and the sponsor. Both the sponsor and FDA may bring consultants to the meeting. The meeting should be directed primarily at establishing agreement between FDA and the sponsor of the overall plan for Phase 3 and the objectives and design of particular studies. The adequacy of the technical information to support Phase 3 studies and/or a marketing application may also be discussed. FDA will also provide its best judgment, at that time, of the pediatric studies that will be required for the drug product and whether their submission will be deferred until after approval. Agreements reached at the meeting on these matters will be recorded in minutes of the conference that will be taken by FDA in accordance with § 10.65 and provided to the sponsor. The minutes along with any other written material provided to the sponsor will serve as a permanent record of any agreements reached. Barring a significant scientific development that requires otherwise, studies conducted in accordance with the agreement shall be presumed to be sufficient in objective and design for the purpose of obtaining marketing approval for the drug.

(2) *"Pre-NDA" and "pre-BLA" meetings.* FDA has found that delays associated with the initial review of a marketing application may be reduced by exchanges of information about a proposed marketing application. The primary purpose of this kind of exchange is to uncover any major unresolved problems, to identify those studies that the sponsor is relying on as adequate and well-controlled to establish the drug's effectiveness, to identify the status of ongoing or needed studies adequate to assess pediatric safety and effectiveness, to acquaint FDA review-

submitted in the marketing application (including technical information), to discuss appropriate methods for statistical analysis of the data, and to discuss the best approach to the presentation and formatting of data in the marketing application. Arrangements for such a meeting are to be initiated by the sponsor with the division responsible for review of the IND. To permit FDA to provide the sponsor with the most useful advice on preparing a marketing application, the sponsor should submit to FDA's reviewing division at least 1 month in advance of the meeting the following information:

(i) A brief summary of the clinical studies to be submitted in the application.

(ii) A proposed format for organizing the submission, including methods for presenting the data.

(iii) Information on the status of needed or ongoing pediatric studies.

(iv) Any other information for discussion at the meeting.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990; 63 FR 66669, Dec. 2, 1998]

§ 312.48 Dispute resolution.

(a) *General.* The Food and Drug Administration is committed to resolving differences between sponsors and FDA reviewing divisions with respect to requirements for IND's as quickly and amicably as possible through the cooperative exchange of information and views.

(b) *Administrative and procedural issues.* When administrative or procedural disputes arise, the sponsor should first attempt to resolve the matter with the division in FDA's Center for Drug Evaluation and Research or Center for Biologics Evaluation and Research which is responsible for review of the IND, beginning with the consumer safety officer assigned to the application. If the dispute is not resolved, the sponsor may raise the matter with the person designated as ombudsman, whose function shall be to investigate what has happened and to facilitate a timely and equitable resolution. Appropriate issues to be dealt with by the ombudsman include resolving difficulties in scheduling meetings and obtaining timely replies to inquiries. Further details on this procedure are contained in FDA Staff Manual Guide 4820.7 that is publicly available under FDA's public information regulations in part 20.

(c) *Scientific and medical disputes.* (1) When scientific or medical disputes arise during the drug investigation process, sponsors should discuss the matter directly with the responsible reviewing officials. If necessary, sponsors may request a meeting with the appropriate reviewing officials and management representatives in order to seek a resolution. Requests for such meetings shall be directed to the director of the division in FDA's Center for Drug Evaluation and Research or Center for Biologics Evaluation and Research which is responsible for review of the IND. FDA will make every attempt to grant requests for meetings that involve important issues and that can be scheduled at mutually convenient times.

(2) The "end-of-Phase 2" and "pre-NDA" meetings described in §312.47(b) will also provide a timely forum for discussing and resolving scientific and medical issues on which the sponsor disagrees with the agency.

(3) In requesting a meeting designed to resolve a scientific or medical dispute, applicants may suggest that FDA seek the advice of outside experts, in which case FDA may, in its discretion, invite to the meeting one or more of its advisory committee members or other consultants, as designated by the agency. Applicants may rely on, and may bring to any meeting, their own consultants. For major scientific and medical policy issues not resolved by informal meetings, FDA may refer the matter to one of its standing advisory committees for its consideration and recommendations.

[52 FR 8831, Mar. 19, 1987, as amended at 55 FR 11580, Mar. 29, 1990]

Subpart D—Responsibilities of Sponsors and Investigators

§ 312.50 General responsibilities of sponsors.

Sponsors are responsibile for selecting qualified investigators, providing them with the information they need to conduct an investigation properly, ensuring proper monitoring of the investigation(s), ensuring that the investigation(s) is conducted in accordance with the general investigational plan and protocols contained in the IND, maintaining an effective IND with respect to the investigations, and ensuring that FDA and all participating investigators are promptly informed of significant new adverse effects or risks with respect to the drug. Additional specific responsibilities of sponsors are described elsewhere in this part.

§ 312.52 Transfer of obligations to a contract research organization.

(a) A sponsor may transfer responsibility for any or all of the obligations set forth in this part to a contract research organization. Any such transfer shall be described in writing; if not all obligations are transferred, the writing is required to describe each of the obligations being assumed by the contract research organization. If all obligations are transferred, a general statement that all obligations have been transferred is acceptable. Any obligation not covered by the written description shall be deemed not to have been transferred.

(b) A contract research organization that assumes any obligation of a sponsor shall comply with the specific regulations in this chapter applicable to this obligation and shall be subject to the same regulatory action as a sponsor for failure to comply with any obligation assumed under these regulations. Thus, all references to "sponsor" in this part apply to a contract research organization to the extent that it assumes one or more obligations of the sponsor.

§ 312.53 Selecting investigators and monitors.

(a) *Selecting investigators.* A sponsor shall select only investigators qualified by training and experience as appropriate experts to investigate the drug.

(b) *Control of drug.* A sponsor shall ship investigational new drugs only to investigators participating in the investigation.

(c) *Obtaining information from the investigator.* Before permitting an investigator to begin participation in an investigation, the sponsor shall obtain the following:

(1) A signed investigator statement (Form FDA-1572) containing:

(i) The name and address of the investigator;

(ii) The name and code number, if any, of the protocol(s) in the IND identifying the study(ies) to be conducted by the investigator;

(iii) The name and address of any medical school, hospital, or other research facility where the clinical investigation(s) will be conducted;

(iv) The name and address of any clinical laboratory facilities to be used in the study;

(v) The name and address of the IRB that is responsible for review and approval of the study(ies);

(vi) A commitment by the investigator that he or she:

(*a*) Will conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, the rights, or welfare of subjects;

(*b*) Will comply with all requirements regarding the obligations of clinical investigators and all other pertinent requirements in this part;

(*c*) Will personally conduct or supervise the described investigation(s);

(*d*) Will inform any potential subjects that the drugs are being used for investigational purposes and will ensure that the requirements relating to obtaining informed consent (21 CFR part 50) and institutional review board review and approval (21 CFR part 56) are met;

(*e*) Will report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with §312.64;

(*f*) Has read and understands the information in the investigator's brochure, including the potential risks and side effects of the drug; and

(*g*) Will ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

(vii) A commitment by the investigator that, for an investigation

to an institutional review requirement under part 56, an IRB that complies with the requirements of that part will be responsible for the initial and continuing review and approval of the clinical investigation and that the investigator will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to the human subjects.

(viii) A list of the names of the subinvestigators (e.g., research fellows, residents) who will be assisting the investigator in the conduct of the investigation(s).

(2) *Curriculum vitae.* A curriculum vitae or other statement of qualifications of the investigator showing the education, training, and experience that qualifies the investigator as an expert in the clinical investigation of the drug for the use under investigation.

(3) *Clinical protocol.* (i) For Phase 1 investigations, a general outline of the planned investigation including the estimated duration of the study and the maximum number of subjects that will be involved.

(ii) For Phase 2 or 3 investigations, an outline of the study protocol including an approximation of the number of subjects to be treated with the drug and the number to be employed as controls, if any; the clinical uses to be investigated; characteristics of subjects by age, sex, and condition; the kind of clinical observations and laboratory tests to be conducted; the estimated duration of the study; and copies or a description of case report forms to be used.

(4) *Financial disclosure information.* Sufficient accurate financial information to allow the sponsor to submit complete and accurate certification or disclosure statements required under part 54 of this chapter. The sponsor shall obtain a commitment from the clinical investigator to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

(d) *Selecting monitors.* A sponsor shall select a monitor qualified by training and experience to monitor the progress of the investigation.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 61 FR 57280, Nov. 5, 1996; 63 FR 5252, Feb. 2, 1998]

§ 312.54 Emergency research under § 50.24 of this chapter.

(a) The sponsor shall monitor the progress of all investigations involving an exception from informed consent under § 50.24 of this chapter. When the sponsor receives from the IRB information concerning the public disclosures required by § 50.24(a)(7)(ii) and (a)(7)(iii) of this chapter, the sponsor promptly shall submit to the IND file and to Docket Number 95S-0158 in the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857, copies of the information that was disclosed, identified by the IND number.

(b) The sponsor also shall monitor such investigations to identify when an IRB determines that it cannot approve the research because it does not meet the criteria in the exception in § 50.24(a) of this chapter or because of other relevant ethical concerns. The sponsor promptly shall provide this information in writing to FDA, investigators who are asked to participate in this or a substantially equivalent clinical investigation, and other IRB's that are asked to review this or a substantially equivalent investigation.

[61 FR 51530, Oct. 2, 1996]

§ 312.55 Informing investigators.

(a) Before the investigation begins, a sponsor (other than a sponsor-investigator) shall give each participating clinical investigator an investigator brochure containing the information described in § 312.23(a)(5).

(b) The sponsor shall, as the overall investigation proceeds, keep each participating investigator informed of new observations discovered by or reported to the sponsor on the drug, particularly with respect to adverse effects and safe use. Such information may be distributed to investigators by means of periodically revised investigator brochures, reprints or published studies, reports or letters to clinical investigators, or other appropriate means. Important safety information is required to be relayed to investigators in accordance with § 312.32.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.56 Review of ongoing investigations.

(a) The sponsor shall monitor the progress of all clinical investigations being conducted under its IND.

(b) A sponsor who discovers that an investigator is not complying with the signed agreement (Form FDA-1572), the general investigational plan, or the requirements of this part or other applicable parts shall promptly either secure compliance or discontinue shipments of the investigational new drug to the investigator and end the investigator's participation in the investigation. If the investigator's participation is to be investigation is ended, the sponsor shall require that the investigator dispose of or return the investigational drug in accordance with the requirements of § 312.59 and shall notify FDA.

(c) The sponsor shall review and evaluate the evidence relating to the safety and effectiveness of the drug as it is obtained from the investigator. The sponsors shall make such reports to FDA regarding information relevant to the safety of the drug as are required under § 312.32. The sponsor shall make annual reports on the progress of the investigation in accordance with § 312.33.

(d) A sponsor who determines that its investigational drug presents an unreasonable and significant risk to subjects shall discontinue those investigations that present the risk, notify FDA, all institutional review boards, and all investigators who have at any time participated in the investigation of the discontinuance, assure the disposition of all stocks of the drug outstanding as required by § 312.59, and furnish FDA with a full report of the sponsor's actions. The sponsor shall discontinue the investigation as soon as possible, and in no event later than 5 working days after making the determination that the investigation should be discontinued. Upon request, FDA will confer with a sponsor on the need to discontinue an investigation.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.57 Recordkeeping and record retention.

(a) A sponsor shall maintain adequate records showing the receipt, shipment, or other disposition of the investigational drug. These records are required to include, as appropriate, the name of the investigator to whom the drug is shipped, and the date, quantity, and batch or code mark of each such shipment.

(b) A sponsor shall maintain complete and accurate records showing any financial interest in § 54.4(a)(3)(i), (a)(3)(ii), (a)(3)(iii), and (a)(3)(iv) of this chapter paid to clinical investigators by the sponsor of the covered study. A sponsor shall also maintain complete and accurate records concerning all other financial interests of investigators subject to part 54 of this chapter.

(c) A sponsor shall retain the records and reports required by this part for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified.

(d) A sponsor shall retain reserve samples of any test article and reference standard identified in, and used in any of the bioequivalence or bioavailability studies described in, § 320.38 or § 320.63 of this chapter, and release the reserve samples to FDA upon request, in accordance with, and for the period specified in § 320.38.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 58 FR 25926, Apr. 28, 1993; 63 FR 5252, Feb. 2, 1998]

§ 312.58 Inspection of sponsor's records and reports.

(a) *FDA inspection.* A sponsor shall upon request from any properly authorized officer or employee of the Food and Drug Administration, at reasonable times, permit such officer or employee to have access to and copy and verify any records and reports relating to a clinical investigation conducted under this part. Upon written request by FDA, the sponsor shall submit the records or reports (or copies of them) to FDA. The sponsor shall discontinue shipments of the drug to any investigator who has failed to maintain or make available records or reports of the investigation as required by this part.

(b) *Controlled substances.* If an investigational new drug is a substance listed in any schedule of the Controlled Substances Act (21 U.S.C. 801; 21 CFR part 1308), records concerning shipment, delivery, receipt, and disposition of the drug, which are required to be kept under this part or other applicable parts of this chapter shall, upon the request of a properly authorized employee of the Drug Enforcement Administration of the U.S. Department of Justice, be made available by the investigator or sponsor to whom the request is made, for inspection and copying. In addition, the sponsor shall assure that adequate precautions are taken, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

§ 312.59 Disposition of unused supply of investigational drug.

The sponsor shall assure the return of all unused supplies of the investigational drug from each individual investigator whose participation in the investigation is discontinued or terminated. The sponsor may authorize alternative disposition of unused supplies of the investigational drug provided this alternative disposition does not expose human subjects to risks from the drug. The sponsor shall maintain written records of any disposition of the drug in accordance with § 312.57.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.60 General responsibilities of investigators.

An investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation. An investigator shall, in accordance with the provisions of part 50 of this chapter, obtain the informed consent of each human subject to whom the drug is administered, except as provided in §§ 50.23 or 50.24 of this chapter. Additional specific responsibilities of clinical investigators are set forth in this part and in parts 50 and 56 of this chapter.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51530, Oct. 2, 1996]

§ 312.61 Control of the investigational drug.

An investigator shall administer the drug only to subjects under the investigator's personal supervision or under the supervision of a subinvestigator responsible to the investigator. The investigator shall not supply the investigational drug to any person not authorized under this part to receive it.

§ 312.62 Investigator recordkeeping and record retention.

(a) *Disposition of drug.* An investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects. If the investigation is terminated, suspended, discontinued, or completed, the investigator shall return the unused supplies of the drug to the sponsor, or otherwise provide for disposition of the unused supplies of the drug under § 312.59.

(b) *Case histories.* An investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

(c) *Record retention.* An investigator shall retain records required to be maintained under this part for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 61 FR 57280, Nov. 5, 1996]

§ 312.64 Investigator reports.

(a) *Progress reports.* The investigator shall furnish all reports to the sponsor of the drug who is responsible for collecting and evaluating the results obtained. The sponsor is required under § 312.33 to submit annual reports to FDA on the progress of the clinical investigations.

(b) *Safety reports.* An investigator shall promptly report to the sponsor any adverse effect that may reasonably be regarded as caused by, or probably caused by, the drug. If the adverse effect is alarming, the investigator shall report the adverse effect immediately.

(c) *Final report.* An investigator shall provide the sponsor with an adequate report shortly after completion of the investigator's participation in the investigation.

(d) *Financial disclosure reports.* The clinical investigator shall provide the sponsor with sufficient accurate financial information to allow an applicant to submit complete and accurate certification or disclosure statements as required under part 54 of this chapter. The clinical investigator shall promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 63 FR 5252, Feb. 2, 1998]

§ 312.66 Assurance of IRB review.

An investigator shall assure that an IRB that complies with the requirements set forth in part 56 will be responsible for the initial and continuing review and approval of the proposed clinical study. The investigator shall also assure that he or she will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risk to human subjects or others, and that he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.68 Inspection of investigator's records and reports.

An investigator shall upon request from any properly authorized officer or employee of FDA, at reasonable times, permit such officer or employee to have access to, and copy and verify any records or reports made by the investigator pursuant to § 312.62. The investigator is not required to divulge subject names unless the records of particular individuals require a more detailed study of the cases, or unless there is reason to believe that the records do not represent actual case studies, or do not represent actual results obtained.

§ 312.69 Handling of controlled substances.

If the investigational drug is subject to the Controlled Substances Act, the

investigator shall take adequate precautions, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

§ 312.70 Disqualification of a clinical investigator.

(a) If FDA has information indicating that an investigator (including a sponsor-investigator) has repeatedly or deliberately failed to comply with the requirements of this part, part 50, or part 56 of this chapter, or has submitted to FDA or to the sponsor false information in any required report, the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research will furnish the investigator written notice of the matter complained of and offer the investigator an opportunity to explain the matter in writing, or, at the option of the investigator, in an informal conference. If an explanation is offered but not accepted by the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research, the investigator will be given an opportunity for a regulatory hearing under part 16 on the question of whether the investigator is entitled to receive investigational new drugs.

(b) After evaluating all available information, including any explanation presented by the investigator, if the Commissioner determines that the investigator has repeatedly or deliberately failed to comply with the requirements of this part, part 50, or part 56 of this chapter, or has deliberately or repeatedly submitted false information to FDA or to the sponsor in any required report, the Commissioner will notify the investigator and the sponsor of any investigation in which the investigator has been named as a participant that the investigator is not entitled to receive investigational drugs. The notification will provide a statement of basis for such determination.

(c) Each IND and each approved application submitted under part 314 containing data r[illegible]ted by an investigator who has [illegible]en determined to be ineligible to receive investigational drugs will be examined to determine whether the investigator has submitted unreliable data that are essential to the continuation of the investigation or essential to the approval of any marketing application.

(d) If the Commissioner determines, after the unreliable data submitted by the investigator are eliminated from consideration, that the data remaining are inadequate to support a conclusion that it is reasonably safe to continue the investigation, the Commissioner will notify the sponsor who shall have an opportunity for a regulatory hearing under part 16. If a danger to the public health exists, however, the Commissioner shall terminate the IND immediately and notify the sponsor of the determination. In such case, the sponsor shall have an opportunity for a regulatory hearing before FDA under part 16 on the question of whether the IND should be reinstated.

(e) If the Commissioner determines, after the unreliable data submitted by the investigator are eliminated from consideration, that the continued approval of the drug product for which the data were submitted cannot be justified, the Commissioner will proceed to withdraw approval of the drug product in accordance with the applicable provisions of the act.

(f) An investigator who has been determined to be ineligible to receive investigational drugs may be reinstated as eligible when the Commissioner determines that the investigator has presented adequate assurances that the investigator will employ investigational drugs solely in compliance with the provisions of this part and of parts 50 and 56.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910 0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23091, June 17, 1987; 55 FR 11580, Mar. 29, 1990; 62 FR 46876, Sept. 5, 1997]

Subpart E—Drugs Intended to Treat Life-threatening and Severely-debilitating Illnesses

AUTHORITY: 21 U.S.C. 351, 352, 353, 355, 371; 42 U.S.C. 262.

SOURCE: 53 FR 41523, Oct. 21, 1988, unless otherwise noted.

§ 312.80 Purpose.

The purpose of this section is to establish procedures designed to expedite the development, evaluation, and marketing of new therapies intended to treat persons with life-threatening and severely-debilitating illnesses, especially where no satisfactory alternative therapy exists. As stated §314.105(c) of this chapter, while the statutory standards of safety and effectiveness apply to all drugs, the many kinds of drugs that are subject to them, and the wide range of uses for those drugs, demand flexibility in applying the standards. The Food and Drug Administration (FDA) has determined that it is appropriate to exercise the broadest flexibility in applying the statutory standards, while preserving appropriate guarantees for safety and effectiveness. These procedures reflect the recognition that physicians and patients are generally willing to accept greater risks or side effects from products that treat life-threatening and severely-debilitating illnesses, than they would accept from products that treat less serious illnesses. These procedures also reflect the recognition that the benefits of the drug need to be evaluated in light of the severity of the disease being treated. The procedure outlined in this section should be interpreted consistent with that purpose.

§ 312.81 Scope.

This section applies to new drug and biological products that are being studied for their safety and effectiveness in treating life-threatening or severely-debilitating diseases.

(a) For purposes of this section, the term "life-threatening" means:

(1) Diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted; and

(2) Diseases or conditions with potentially fatal outcomes, where the end point of clinical trial analysis is survival.

(b) For purposes of this section, the term "severely debilitating" means diseases or conditions that cause major irreversible morbidity.

(c) Sponsors are encouraged to consult with FDA on the applicability of these procedures to specific products.

[53 FR 41523, Oct. 21, 1988, as amended at 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, §312.81 was amended by removing "; antibiotic," from the introductory text, effective May 20, 1999.

§ 312.82 Early consultation.

For products intended to treat life-threatening or severely-debilitating illnesses, sponsors may request to meet with FDA-reviewing officials early in the drug development process to review and reach agreement on the design of necessary preclinical and clinical studies. Where appropriate, FDA will invite to such meetings one or more outside expert scientific consultants or advisory committee members. To the extent FDA resources permit, agency reviewing officials will honor requests for such meetings.

(a) *Pre-investigational new drug (IND) meetings.* Prior to the submission of the initial IND, the sponsor may request a meeting with FDA-reviewing officials. The primary purpose of this meeting is to review and reach agreement on the design of animal studies needed to initiate human testing. The meeting may also provide an opportunity for discussing the scope and design of phase 1 testing, plans for studying the drug product in pediatric populations, and the best approach for presentation and formatting of data in the IND.

(b) *End-of-phase 1 meetings.* When data from phase 1 clinical testing are available, the sponsor may again request a meeting with FDA-reviewing officials. The primary purpose of this meeting is to review and reach agreement on the design of phase 2 controlled clinical trials, with the goal that such testing will be adequate to provide sufficient data on the drug's safety and effectiveness to support a decision on its approvability for marketing, and to discuss the need for, as well as the design and timing of, studies of the drug in pediatric patients. For drugs for life-threatening diseases, FDA will provide its best judgment, at that time, whether pediatric studies will be required and whether their submission will be

deferred until after approval. The procedures outlined in §312.47(b)(1) with respect to end-of-phase 2 conferences, including documentation of agreements reached, would also be used for end-of-phase 1 meetings.

[53 FR 41523, Oct. 21, 1988, as amended at 63 FR 66669, Dec. 2, 1998]

§ 312.83 Treatment protocols.

If the preliminary analysis of phase 2 test results appears promising, FDA may ask the sponsor to submit a treatment protocol to be reviewed under the procedures and criteria listed in §§312.34 and 312.35. Such a treatment protoco' if requested and granted, would normally remain in effect while the complete data necessary for a marketing application are being assembled by the sponsor and reviewed by FDA (unless grounds exist for clinical hold of ongoing protocols, as provided in §312.42(b)(3)(ii)).

§ 312.84 Risk-benefit analysis in review of marketing applications for drugs to treat life-threatening and severely-debilitating illnesses.

(a) FDA's application of the statutory standards for marketing approval shall recognize the need for a medical risk-benefit judgment in making the final decision on approvability. As part of this evaluation, consistent with the statement of purpose in §312.80, FDA will consider whether the benefits of the drug outweigh the known and potential risks of the drug and the need to answer remaining questions about risks and benefits of the drug, taking into consideration the severity of the disease and the absence of satisfactory alternative therapy.

(b) In making decisions on whether to grant marketing approval for products that have been the subject of an end-of-phase 1 meeting under §312.82, FDA will usually seek the advice of outside expert scientific consultants or advisory committees. Upon the filing of such a marketing application under 314.101 or part 601 of this chapter, FDA will notify the members of the relevant standing advisory committee of the application's filing and its availability for review.

(c) If FDA concludes that the data presented are not sufficient for marketing approval, FDA will issue (for a drug) a not approvable letter pursuant to §314.120 of this chapter, or (for a biologic) a deficiencies letter consistent with the biological product licensing procedures. Such letter, in describing the deficiencies in the application, will address why the results of the research design agreed to under §312.82, or in subsequent meetings, have not provided sufficient evidence for marketing approval. Such letter will also describe any recommendations made by the advisory committee regarding the application.

(d) Marketing applications submitted under the procedures contained in this section will be subject to the requirements and procedures contained in part 314 or part 600 of this chapter, as well as those in this subpart.

§ 312.85 Phase 4 studies.

Concurrent with marketing approval, FDA may seek agreement from the sponsor to conduct certain postmarketing (phase 4) studies to delineate additional information about the drug's risks, benefits, and optimal use. These studies could include, but would not be limited to, studying different doses or schedules of administration than were used in phase 2 studies, use of the drug in other patient populations or other stages of the disease, or use of the drug over a longer period of time.

§ 312.86 Focused FDA regulatory research.

At the discretion of the agency, FDA may undertake focused regulatory research on critical rate-limiting aspects of the preclinical, chemical/manufacturing, and clinical phases of drug development and evaluation. When initiated, FDA will undertake such research efforts as a means for meeting a public health need in facilitating the development of therapies to treat life-threatening or severely debilitating illnesses.

§ 312.87 Active monitoring of conduct and evaluation of clinical trials.

For drugs covered under this section, the Commissioner and other agency officials will monitor the progress of the conduct and evaluation of clinical trials and be involved in facilitating their appropriate progress.

§ 312.88 Safeguards for patient safety.

All of the safeguards incorporated within parts 50, 56, 312, 314, and 600 of this chapter designed to ensure the safety of clinical testing and the safety of products following marketing approval apply to drugs covered by this section. This includes the requirements for informed consent (part 50 of this chapter) and institutional review boards (part 56 of this chapter). These safeguards further include the review of animal studies prior to initial human testing (§312.23), and the monitoring of adverse drug experiences through the requirements of IND safety reports (§312.32), safety update reports during agency review of a marketing application (§314.50 of this chapter), and postmarketing adverse reaction reporting (§314.80 of this chapter).

Subpart F—Miscellaneous

§ 312.110 Import and export requirements.

(a) *Imports.* An investigational new drug offered for import into the United States complies with the requirements of this part if it is subject to an IND that is in effect for it under §312.40 and: (1) The consignee in the United States is the sponsor of the IND; (2) the consignee is a qualified investigator named in the IND; or (3) the consignee is the domestic agent of a foreign sponsor, is responsible for the control and distribution of the investigational drug, and the IND identifies the consignee and describes what, if any, actions the consignee will take with respect to the investigational drug.

(b) *Exports.* An investigational new drug intended for export from the United States complies with the requirements of this part as follows:

(1) If an IND is in effect for the drug under §312.40 and each person who receives the drug is an investigator named in the application; or

(2) If FDA authorizes shipment of the drug for use in a clinical investigation. Authorization may be obtained as follows:

Through submission to the International Affairs Staff (HFY-50), Associate Commissioner for Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, of a written request from the person that seeks to export the drug. A request must provide adequate information about the drug to satisfy FDA that the drug is appropriate for the proposed investigational use in humans, that the drug will be used for investigational purposes only, and that the drug may be legally used by that consignee in the importing country for the proposed investigational use. The request shall specify the quantity of the drug to be shipped per shipment and the frequency of expected shipments. If FDA authorizes exportation under this paragraph, the agency shall concurrently notify the government of the importing country of such authorization.

(ii) Through submission to the International Affairs Staff (HFY-50), Associate Commissioner for Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, of a formal request from an authorized official of the government of the country to which the drug is proposed to be shipped. A request must specify that the foreign government has adequate information about the drug and the proposed investigational use, that the drug will be used for investigational purposes only, and that the foreign government is satisfied that the drug may legally be used by the intended consignee in that country. Such a request shall specify the quantity of drug to be shipped per shipment and the frequency of expected shipments.

(iii) Authorization to export an investigational drug under paragraph (b)(2)(i) or (ii) of this section may be revoked by FDA if the agency finds that the conditions underlying its authorization are not longer met.

(3) This paragraph applies only where the drug is to be used for the purpose of clinical investigation.

(4) This paragraph does not apply to the export of new drugs (including biological products, antibiotic drugs, and insulin) approved or authorized for export under section 802 of the Act (21 U.S.C. 382), or section 351(h)(1)(A) of the

Public Health Service Act (42 U.S.C. 262(h)(1)(A)).

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, §312.110 was amended by revising paragraph (b)(4) and by removing paragraph (b)(5), effective May 20, 1999. For the convenience of the user, the superseded text follows:

§312.110 Import and export requirements.

* * * * *

(b) * * *

(4) This paragraph does not apply to the export of an antibiotic drug product shipped in accordance with the provisions of section 801(d) of the act.

(5) This paragraph does not apply to the export of new drugs (including biological products) approved for export under section 802 of the act or section 351(h)(1)(A) of the Public Health Service Act.

* * * * *

§312.120 Foreign clinical studies not conducted under an IND.

(a) *Introduction.* This section describes the criteria for acceptance by FDA of foreign clinical studies not conducted under an IND. In general, FDA accepts such studies provided they are well designed, well conducted, performed by qualified investigators, and conducted in accordance with ethical principles acceptable to the world community. Studies meeting these criteria may be utilized to support clinical investigations in the United States and/or marketing approval. Marketing approval of a new drug based solely on foreign clinical data is governed by §314.106.

(b) *Data submissions.* A sponsor who wishes to rely on a foreign clinical study to support an IND or to support an application for marketing approval shall submit to FDA the following information:

(1) A description of the investigator's qualifications;

(2) A description of the research facilities;

(3) A detailed summary of the protocol and results of the study, and, should FDA request, case records maintained by the investigator or additional background data such as hospital or other institutional records;

(4) A description of the drug substance and drug product used in the study, including a description of components, formulation, specifications, and bioavailability of the specific drug product used in the clinical study, if available; and

(5) If the study is intended to support the effectiveness of a drug product, information showing that the study is adequate and well controlled under §314.126.

(c) *Conformance with ethical principles.* (1) Foreign clinical research is required to have been conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" (see paragraph (c)(4) of this section) or the laws and regulations of the country in which the research was conducted, whichever represents the greater protection of the individual.

(2) For each foreign clinical study submitted under this section, the sponsor shall explain how the research conformed to the ethical principles contained in the "Declaration of Helsinki" or the foreign country's standards, whichever were used. If the foreign country's standards were used, the sponsor shall explain in detail how those standards differ from the "Declaration of Helsinki" and how they offer greater protection.

(3) When the research has been approved by an independent review committee, the sponsor shall submit to FDA documentation of such review and approval, including the names and qualifications of the members of the committee. In this regard, a "review committee" means a committee composed of scientists and, where practicable, individuals who are otherwise qualified (e.g., other health professionals or laymen). The investigator may not vote on any aspect of the review of his or her protocol by a review committee.

(4) The "Declaration of Helsinki" states as follows:

RECOMMENDATIONS GUIDING PHYSICIANS IN BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

Introduction

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. Basic Principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be

dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. Medical Research Combined with Professional Care (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, reestablishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient—including those of a control group, if any—should be assured of the best proven diagnostic and therapeutic method.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. Non-Therapeutic Biomedical Research Involving Human Subjects (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subjects should be volunteers—either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 56 FR 22113, May 14, 1991; 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, §312.120 was amended by removing "or antibiotic drug" from the last sentence of paragraph (a), effective May 20, 1999.

§312.130 Availability for public disclosure of data and information in an IND.

(a) The existence of an investigational new drug application will not be disclosed by FDA unless it has previously been publicly disclosed or acknowledged.

(b) The availability for public disclosure of all data and information in an investigational new drug application for a new drug will be handled in accordance with the provisions established in §314.430 for the confidentiality of data and information in applications submitted in part 314. The availability for public disclosure of all data and information in an investigational new drug application for a biological product will be governed by the provisions of §§601.50 and 601.51.

(c) Notwithstanding the provisions of §314.430, FDA shall disclose upon request to an individual to whom an investigational new drug has been given a copy of any IND safety report relating to the use in the individual.

(d) The availability of information required to be publicly disclosed for investigations involving an exception from informed consent under §50.24 of this chapter will be handled as follows: Persons wishing to request the publicly disclosable information in the IND that was required to be filed in Docket Number 95S-0158 in the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857, shall submit a request under the Freedom of Information Act.

[52 FR 8831, Mar. 19, 1987. Redesignated at 53 FR 41523, Oct. 21, 1988, as amended at 61 FR 51530, Oct. 2, 1996; 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, §312.130 was amended by removing "or antibiotic drug" from paragraph (b), effective May 20, 1999.

§312.140 Address for correspondence.

(a) Except as provided in paragraph (b) of this section, a sponsor shall send an initial IND submission to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, Park Bldg., Rm. 214, 12420 Parklawn Dr., Rockville, MD 20852. On receiving the IND, FDA will inform the sponsor which one of the divisions in the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research is responsible for the IND. Amendments, reports, and other correspondence relating to matters covered by the IND should be directed to the appropriate division. The outside wrapper of each submission shall state what is contained in the submission, for example, "IND Application", "Protocol Amendment", etc.

(b) Applications for the products listed below should be submitted to the Division of Biological Investigational New Drugs (HFB-230), Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892. (1) Products subject to the licensing provisions of the Public Health Service Act of July 1, 1944 (58 Stat. 682, as amended (42 U.S.C. 201 *et seq.*)) or subject to part 600; (2) ingredients packaged together with containers intended for the collection, processing, or storage of blood or blood components; (3) urokinase products; (4) plasma volume expanders and hydroxyethyl starch for leukapheresis; and (5) coupled antibodies, i.e., products that consist of an antibody component coupled with a drug or radionuclide component in which both components provide a pharmacological effect but the biological component de-[illegible]

(c) All correspondence relating to biological products for human use which are also radioactive drugs shall be submitted to the Division of Oncology and Radiopharmaceutical Drug Products (HFD-150), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, except that applications for coupled antibodies shall be submitted in accordance with paragraph (b) of this section.

(d) All correspondence relating to export of an investigational drug under §312.110(b)(2) shall be submitted to the International Affairs Staff (HFY-50), Office of Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990]

§312.145 Guidelines.

(a) FDA has made available guidelines under §10.90(b) to help persons to comply with certain requirements of this part.

(b) The Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research maintain lists of guidelines that apply to the Centers' regulations. The lists state how a person can obtain a copy of each guideline. A request for a copy of the lists should be directed to the CDER Executive Secretariat Staff (HFD-8), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, for drug products, and the Congressional, Consumer, and International Affairs Staff (HFB-142), Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892, for biological products.

[52 FR 8831, Mar. 19, 1987, as amended at 55 FR 11580, Mar. 29, 1990; 56 FR [illegible] Jan. 31, 1991; 57 FR 10814, Mar. 31, 1992]

Subpart G—Drugs for Investigational Use in Laboratory Research Animals or In Vitro Tests

§312.160 Drugs for investigational use in laboratory research animals or in vitro tests.

(a) *Authorization to ship.* (1)(i) A person may ship a drug intended solely for tests in vitro or in animals used only for laboratory research purposes if it is labeled as follows:

CAUTION: Contains a new drug for investigational use only in laboratory research animals, or for tests in vitro. Not for use in humans.

(ii) A person may ship a biological product for investigational in vitro diagnostic use that is listed in §312.2(b)(2)(ii) if it is labeled as follows:

CAUTION: Contains a biological product for investigational in vitro diagnostic tests only.

(2) A person shipping a drug under paragraph (a) of this section shall use due diligence to assure that the consignee is regularly engaged in conducting such tests and that the shipment of the new drug will actually be used for tests in vitro or in animals used only for laboratory research.

(3) A person who ships a drug under paragraph (a) of this section shall maintain adequate records showing the name and post office address of the expert to whom the drug is shipped and the date, quantity, and batch or code mark of each shipment and delivery. Records of shipments under paragraph (a)(1)(i) of this section are to be maintained for a period of 2 years after the shipment. Records and reports of data and shipments under paragraph (a)(1)(ii) of this section are to be maintained in accordance with §312.57(b). The person who ships the drug shall upon request from any properly authorized officer or employee of the Food and Drug Administration, at reasonable times, permit such officer or employee to have access to and copy and verify records required to be maintained under this section.

(b) *Termination of authorization to ship.* FDA may terminate authorization to ship a drug under this section if it finds that:

(1) The sponsor of the investigation has failed to comply with any of the conditions for shipment established under this section; or

(2) The continuance of the investigation is unsafe or otherwise contrary to the public interest or the drug is used for purposes other than bona fide scientific investigation. FDA will notify the person shipping the drug of its finding and invite immediate correction. If correction is not immediately made, the person shall have an opportunity for a regulatory hearing before FDA pursuant to part 16.

(c) *Disposition of unused drug.* The person who ships the drug under paragraph (a) of this section shall assure the return of all unused supplies of the drug from individual investigators whenever the investigation discontinues or the investigation is terminated. The person who ships the drug may authorize in writing alternative disposition of unused supplies of the drug provided this alternative disposition does not expose humans to risks from the drug, either directly or indirectly (e.g., through food-producing animals). The shipper shall maintain records of any alternative disposition.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987. Redesignated at 53 FR 41523, Oct. 21, 1988]

PART 314—APPLICATIONS FOR FDA APPROVAL TO MARKET A NEW DRUG

Subpart A—General Provisions

Subpart B—Applications

Subpart C—Abbreviated Applications

Subpart D—FDA Action on Applications and Abbreviated Applications

Subpart E—Hearing Procedures for New Drugs

Subpart F—Administrative Procedures for Antibiotics

Subpart G—Miscellaneous Provisions

Subpart H—Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses

AUTHORITY: 21 U.S.C. 321, 331, 351, 352, 353, 355, 371, 374, 379e.

SOURCE: 50 FR 7493, Feb. 22, 1985, unless

§50.20 General requirements for informed consent.

Except as provided in §50.23, no investigator may involve a human being as a subject in research covered by these regulations unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative. An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject or the representative shall be in language understandable to the subject or the representative. No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution, or its agents from liability for negligence.

§50.25 Elements of informed consent.

(a) *Basic elements of informed consent.* In seeking informed consent, the following information shall be provided to each subject:

(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental.

(2) A description of any reasonably foreseeable risks or discomforts to the subject.

(3) A description of any benefits to the subject or to others which may reasonably be expected from the research.

(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject.

(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that the Food and Drug Administration may inspect the records.

(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained.

(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject.

(8) A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

(b) *Additional elements of informed consent.* When appropriate, one or more of the following elements of information shall also be provided to each subject:

(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) which are currently unforeseeable.

(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent.

(3) Any additional costs to the subject that may result from participation in the research.

(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.

(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject.

(6) The approximate number of subjects involved in the study.

(c) The informed consent requirements in these regulations are not intended to preempt any applicable Federal, State, or local laws which require additional information to be disclosed for informed consent to be legally effective.

(d) Nothing in these regulations is intended to limit the authority of a physician to provide emergency medical care to the extent the physician is permitted to do so under applicable Federal, State, or local law.

Information Sheet for a Claim of Categorical
Exclusion for an IND Under 21 CFR 25.24

For those wastes generated in the production and use of the product which will be controlled, please include documentation that such waste storage or disposal is in compliance with federal, state and local requirements for hazardous waste production. As an alternative, identify any generally recognized, scientifically sound control procedures which have been implemented to reduce the likelihood of inadvertent release of potentially toxic materials into the environment (e.g., compliance with the NIH Guidelines for Research Involving Recombinant DNA Molecules [51 FR 16958 (1986)] and/or compliance with the EPA Effluent Guidelines and Standards for Pharmaceutical Manufacturing [40 CFR 439]). If these alternatives are not applicable, a description of the control procedures actually used to prevent waste from entering the environment should be submitted.

For those wastes generated in the production and use of the product which will not be controlled, please list the potentially toxic waste compounds, including the quantities and concentrations which may be expected to enter the environment from both productions of the product and from the intended clinical studies, and briefly describe the immediate environment into which such release will occur. Further, provide the appropriate references or experimental data from which it may be reasonably concluded that such release is non-toxic.

If the waste to be generated during the production and proposed investigational use of this product is either not controlled or is not reasonably expected to be non-toxic in the environment to which it will be released, please submit an environmental assessment using the format described in 21 CFR 25.31.

If actions under proposed amendments to this IND substantially alter the quantity, quality or conditions of waste release in such a way as to alter the basis for either a claim of categorical exclusion or an environmental assessment, then such amendments should be supported by the appropriate data for a claim of categorical exclusion or an amended environmental assessment for wastes generated under the proposed amendments to this IND.

An investigator sponsored IND for which no additional product manufacturing is intended will ordinarily have addressed these environmental issues by incorporating the manufacturer's IND or MF by cross reference. However, if the use of the product during clinical investigation is expected to result in the uncontrolled release of toxic materials into the environment then an environmental assessment should be submitted.

3/30/94

7

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

Our STN: BL [125377/0]

BLA ACKNOWLEDGEMENT
July 8, 2010

Bristol-Myers Squibb Company
Attention: A. Heather Knight-Trent, Pharm D.
Director-Oncology
5 Research Parkway
Wallingford, CT 06492-7660

Dear Dr. Knight-Trent:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act (PHS Act) for the following:

Name of Biological Product: Ipilimumab

Date of Application: JUNE 25, 2010

Date of Receipt: JUNE 25, 2010

Our Submission Tracking Number (STN): BL 125377/0

Proposed Use: Pretreated Advanced Melanoma

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

The BLA Submission Tracking Number provided above should be cited at the top of the first page of all submissions to this application. If you have any questions, contact Erik S. Laughner, Senior Regulatory Health Project Manager, at (301) 796-1393.

Sincerely,

/Patricia Keegan/
Patricia Keegan, M.D.
Director
Division of Biologic Oncology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Exhibit 8

DESCRIPTION OF SIGNIFICANT ACTIVITIES OF APPLICANT DURING REGULATORY REVIEW

Date	Event
12-JUL-2000	Submission of initial IND under IND8937.005 by Medarex
13-JUL-2000	FDA receipt of IND (transferred by FDA to IND 9186.000)
31-JUL-2000	Submission of protocol amendment
24-AUG-2000	Submission of protocol IRB approval
27-NOV-2000	Submission of protocol amendment
23-AUG-2001	Submission of protocol amendments and investigator brochure
04-SEP-2001	Submission of annual report
11-MAR-2002	Submission of protocol amendment, IRB approval, approved informed consent, and toxicity report
27-JUN-2002	Submission of pharmacokinetic data
27-JUN-2002	Submission of protocol, DTIC, and informed consent
20-AUG-2002	Submission of protocol amendment
7-OCT-2002	Submission of annual report containing revised investigator brochure
20-JAN-2003	Response to FDA request for monitoring information
28-FEB-2003	Submission of new protocol
21-MAR-2003	Submission of protocol amendment
13-MAY-2003	Request for mid-phase II teleconference
19-MAY-2003	Submission of protocol
01-JUL-2003	Submission of new protocol
03-JUL-2003	Submission of protocol amendment
09-JUL-2003	Submission of protocol amendment
05-AUG-2003	Submission of mid-phase II follow-up questions
11-SEP-2003	Response to FDA request regarding patient death
16-SEP-2003	Submission of protocol amendment
06-OCT-2003	Submission of annual report
23-DEC-2003	Submission of investigator brochure, version 4

Date	Event
29-DEC-2003	Request for EOP2 meeting
10-MAR-2004	Submission of protocol amendment
17-MAY-2004	Submission of protocol amendment
03-JUN-2004	Submission of protocol amendment
22-JUN-2004	Submission of protocol amendment
22-JUN-2004	Submission of protocol amendment
20-AUG-2004	Submission of annual report
18-OCT-2004	Submission of investigator brochure, version 5
27-OCT-2004	Submission of protocol amendment
01-JAN-2005	Submission of annual report supplement and investigator brochure, version 6
04-MAR-2005	Submission of protocol amendment
22-MAR-2005	Submission of quarterly safety report
28-MAR-2005	Submission of rational for protocol arm
20-JUL-2005	Notification of study closure for Canadian protocol
28-JUL-2005	Submission of quarterly safety report
08-AUG-2005	Transfer of IND from Medarex to Bristol Myers Squibb (BMS)
11-AUG-2005	FDA acceptance of BMS sponsorship
23-AUG-2005	Submission of investigator brochure
30-AUG-2005	Notification of orphan drug designation
12-SEPT-2005	Submission of new investigator information
13-SEP-2005	Submission of data summary
22-SEP-2005	Submission of quarterly safety report
26-SEP-2005	Request for type B meeting
03-OCT-2005	Submission of protocol amendment
05-OCT-2005	Submission of preliminary safety data, protocol synopsis, and draft protocol
06-OCT-2005	Receipt of Orphan Drug Application letter from FDA
13-OCT-2005	FDA acceptance of sponsorship
14-OCT-2005	Confirmation from FDA of EOP1 meeting
20-OCT-2005	Submission of annual report
20-OCT-2005	Submission of investigator brochure, version 8
28-OCT-2005	FDA comments on briefing document submitted for EOP1 meeting

Date	Event
03-NOV-2005	Request for SPA
18-NOV-2005	Submission of protocol amendments, revised protocol, and new investigator information
22-NOV-2005	Submission updated safety data
22-NOV-2005	Preliminary comments from FDA regarding BMS questions
29-NOV-2005	Submission of CMC amendment
07-DEC-2005	FDA minutes of 11/28/05 EOP1/pre-phase 2 meeting
09-DEC-2005	Correspondence regarding sponsor minutes from 11/28/05 EOP1 meeting and FDA comments on SPA
13-DEC-2005	Submission of protocol amendments and new investigator information
14-DEC-2005	Submission of quarterly safety report
22-DEC-2005	Synchronization of oncology annual reports Submission of annual integrated summary of safety
11-JAN-2006	Submission of protocol amendments
26-JAN-2006	Request for FDA feedback and guidance on revision of protocol
30-JAN-2006	FDA responses to BMS questions regarding protocol revisions
06-FEB-2006	Request for SPA
13-FEB-2006	Protocols for studies to investigate monotherapy in solid tumors or hematologic malignancies can be included under IND 8937.
20-FEB-2006	SPA submission
21-FEB-2006	Submission of new protocol, revised protocol, and protocol amendments
21-FEB-2006	Submission of request for SPA
12-MAR-2006	Discussion of SPA with FDA
14-MAR-2006	Submission of new investigator information
14-MAR-2006	Letter to investigators regarding protocol
15-MAR-2006	FDA request for a formal type C meeting
16-MAR-2006	Request for SPA
16-MAR-2006	FDA rejection of request for SPA
20-MAR-2006	Submission of draft statistical analysis plan
23-MAR-2006	Correspondence from FDA regarding SPA
24-MAR-2006	FDA internal meeting regarding SPA
24-MAR-2006	FDA rejection of request for SPA
29-MAR-2006	Submission of new investigator information

Date	Event
11-APR-2006	Request for SPA for revised protocol
13-APR-2006	Submission of protocol amendments and revised protocol Transfer of obligations to CROs
20-APR-2006	Submission of new investigator information
20-APR-2006	Submission of new protocol Transfer of obligations to CROs
10-MAY-2006	Submission of new investigator information
11-MAY-2006	FDA comments on SPA
15-MAY-2006	FDA grant of SPA
18-MAY-2006	Teleconference regarding reproductive toxicology studies
18-MAY-2006	Request for FDA clarification on SPA
18-MAY-2006	Request for FDA guidance on international Phase 3 trial.
19-MAY-2006	Withdrawal of protocol
24-MAY-2006	Response to FDA regarding reproductive toxicology studies
30-MAY-2006	Resubmission of request for SPA
31-MAY-2006	Submission of administrative letter, protocol amendment, and new investigator information
02-JUN-2006	Submission of administrative letter
14-JUN-2006	Submission of revised protocol
16-JUN-2006	FDA comments on SPA
27-JUN-2006	Submission of BMS proposal regarding reproductive toxicology studies
28-JUN-2006	Submission of annual report
29-JUN-2006	Submission of new investigator information
12-JUL-2006	Request for FDA advice regarding proposed modifications to IRC charters
17-JUL-2006	Submission of protocol amendment and new investigator information
21-JUL-2006	Request for FDA feedback on retreatment recommendation
26-JUL-2006	Submission of new investigator information
03-AUG-2006	Submission of protocol amendment and new investigator information
05-AUG-2006	FDA review of retreatment recommendation
10-AUG-2006	Submission of proposal regarding DMC review
14-AUG-2006	Submission of new investigator information
22-AUG-2006	Submission of new investigator information
25-AUG-2006	Request for CMC type B meeting

Date	Event
28-AUG-2006	Submission of protocol amendment and new investigator information
31-AUG-2006	Receipt of FDA feedback regarding BMS proposal for IRC charters
31-AUG-2006	Teleconference regarding a site-specific amendment
31-AUG-2006	Request for EOP1 type B meeting
07-SEP-2006	Submission of protocol amendment, informed consent form, and draft case report forms
14-SEP-2006	Submission of new investigator information and investigator brochure
18-SEP-2006	Submission of background information for 10/19/06 CMC type B meeting
26-SEP-2006	Submission of new investigator information
03-OCT-2006	BMS request for FDA review of cardiovascular safety and assessments.
06-OCT-2006	BMS request for fast-track designation
12-OCT-2006	FDA comments on site-specific amendment
26-OCT-2006	Submission of protocol amendment and revised protocol
27-OCT-2006	Submission of new investigator information for protocols CA184-022, -025
27-OCT-2006	Submission of new investigator information and revised protocol
03-NOV-2006	Submission of protocol amendments and revised protocols
07-NOV-2006	Follow-up from 10/19/06 CMC type B meeting
08-NOV-2006	Submission of new investigator information
10-NOV-2006	Submission of new investigator information
21-NOV-2006	Submission of clinical study report
21-NOV-2006	Submission of new investigator information
28-NOV-2006	FDA Grant of fast track designation
28-NOV-2006	FDA support for site-specific protocol amendment
07-DEC-2006	Ipilimumab presentation to DMC
13-DEC-2006	Submission of IND SN352
15-DEC-2006	Submission of new investigator information
19-DEC-2006	Submission of new investigator information
21-DEC-2006	Submission of new investigator information
09-JAN-2007	Request to FDA regarding wording for amendments and regarding patients with mixed response
12-JAN-2007	Submission of new investigator information
18-JAN-2007	Briefing of new FDA reviewer on recent expedited safety reports.

Date	Event
02-FEB-2007	Submission of SPA, draft revised protocol, protocol amendment, and draft DMC charter
05-FEB-2007	Submission of SPA, draft revised protocol, informed consent form, case report form, and DMC charter
08-FEB-2007	Submission of new investigator information
08-FEB-2007	Submission of protocol amendment and revised protocol
06-MAR-2007	Issue of investigator letter regarding collecting blood samples.
07-MAR-2007	Submission of administrative letter and investigator letter
15-MAR-2007	Submission of addendum to investigator brochure, version 9
16-MAR-2007	Amendment to Drug Master File for CMC variation for a new presentation
22-MAR-2007	Submission of protocol amendment and revised protocol
27-MAR-2007	Submission to FDA of minutes from 11/2/06 meeting
30-MAR-2007	Submission of administrative letter, protocol amendment, and revised protocol
03-APR-2007	Submission of new investigator information
04-APR-2007	Submission of new protocol
10-APR-2007	Submission of administrative letter
13-APR-2007	Submission of CMC information amendment
20-APR-2007	Proposal of addendum to IRC charter
25-APR-2007	Notice to FDA of a safety event
30-APR-2007	Submission of new investigator information
01-MAY-2007	Submission of initial written report for expedited safety report
11-MAY-2007	Submission of protocol amendment
15-MAY-2007	Response to FDA request for synopsis for ipilimumab treatment use protocol
16-MAY-2007	Submission of new investigator information
22-MAY-2007	Submission of new protocol
24-MAY-2007	Submission of request for review of proposed tradename
25-MAY-2007	Submission of addendum to IRC charter
30-MAY-2007	Submission of new investigator information
04-JUN-2007	Submission of new investigator information
05-JUN-2007	Discussion of timelines for submission of treatment protocol with FDA
11-JUN-2007	Response to FDA request for information
15-JUN-2007	Submission of addendum to investigator brochure

Date	Event
15-JUN-2007	Submission of draft treatment protocol
20-JUN-2007	Submission of administrative letters and revised protocol
20-JUN-2007	Submission of protocol amendment and revised protocol
22-JUN-2007	Pre-BLA meeting discussion with FDA
26-JUN-2007	Submission of annual report
27-JUN-2007	Response to FDA request for information
28-JUN-2007	Submission of new investigator information
29-JUN-2007	BMS to provide Core Statistical Analysis Plan for Clinical Study Reports of Protocols CA184-004, 007, -008, -022 and -024 in Unresectable Stage III or IV Melanoma
03-JUL-2007	Submission of administrative letters
12-JUL-2007	Submission of new investigator information
13-JUL-2007	Submission of revised draft treatment protocol
17-JUL-2007	Submission of new investigator information
17-JUL-2007	Submission of protocol amendment, revised protocol, and informed consent form
17-JUL-2007	Correspondence with FDA regarding SPA
18-JUL-2007	FDA receipt of development meeting background document
18-JUL-2007	FDA approval of draft treatment protocol
18-JUL-2007	Letter from FDA regarding treatment use protocol
18-JUL-2007	Response to FDA request for submission of development meeting background document as an informal amendment to IND
19-JUL-2007	Response to FDA request for redline of revised protocol
20-JUL-2007	Submission of new protocol
31-JUL-2007	Submission of protocol amendment and revised protocols
08-AUG-2007	Submission of new investigator information
08-AUG-2007	Submission of new investigator information
20-AUG-2007	Submission of protocol amendment and revised protocols
20-AUG-2007	Submission of new investigator information
29-AUG-2007	Request for a face-to-face CMC pre-BLA type B meeting
06-SEPT-2007	Submission of new investigator information
07-SEPT-2007	Letter from FDA confirming 10/31/07 type B meeting
25-SEPT-2007	Submission of CMC pre-BLA type B meeting background document
02-OCT-2007	Submission of investigator brochure

Date	Event
04-OCT-2007	Submission of new investigator information
05-OCT-2007	Communication of plans regarding access program
15-OCT-2007	Submission of minutes from 8/28/07 meeting and update on status of communication plan
15-OCT-2007	Request for a type B meeting
16-OCT-2007	Submission of new investigator information
23-OCT-2007	Submission of new investigator information
23-OCT-2007	Submission of communication plan for treatment protocol
29-OCT-2007	Letter from FDA regarding type B meeting
29-OCT-2007	FDA comments on briefing package for 10/31/07 CMC pre-BLA meeting
31-OCT-2007	Type B CMC pre-BLA meeting with FDA
05-NOV-2007	Submission of new investigator information and administrative letter
14-NOV-2007	Submission of new investigator information
14-NOV-2007	Submission of updated consent forms
16-NOV-2007	Submission of protocol amendment and revised protocols
16-NOV-2007	Submission of background document for type B meeting
03-DEC-2007	Submission of new investigator information
05-DEC-2007	Submission of clinical study reports
12-DEC-2007	Response to FDA questions
14-DEC-2007	Submission of new investigator information
19-DEC-2007	Response to FDA questions received on 12/18/07
20-DEC-2007	Type B meeting at FDA
07-JAN-2008	Notification of new BMS contact
24-JAN-2008	Response to FDA request for hepatotoxicity analysis
25-JAN-2008	Submission of new investigator information
31-JAN-2008	FDA response to hepatotoxicity analysis
31-JAN-2008	FDA feedback on review of tradename
04-FEB-2008	Submission of change in investigator information
06-FEB-2008	Email correspondence with FDA regarding hepatotoxicity management
08-FEB-2008	Submission of administrative letter
20-FEB-2008	BMS request for type C meeting
21-FEB-2008	Withdrawal of application for type C meeting.

Date	Event
21-FEB-2008	Request for a face-to-face pre-BLA type B meeting
25-FEB-2008	Submission of final clinical study report
26-FEB-2008	Submission of change in investigator information
27-FEB-2008	Request for SPA
03-MAR-2008	Letter from FDA confirming 4/25/08 pre-BLA meeting
11-MAR-2008	Submission of protocol amendment and revised protocols
21-MAR-2008	Submission of background document for type B pre-BLA meeting.
28-MAR-2008	Notification of new BMS contact
28-MAR-2008	FDA Comments on SPA
01-APR-2008	Response to FDA comments regarding SPA
02-APR-2008	Submission of new investigator information and change in investigator information
11-APR-2008	FDA request regarding expanded access protocol
14-APR-2008	Submission of new protocol and new investigator information
14-APR-2008	Acknowledgement acceptance letter for SPA
15-APR-2008	Submission of changes in investigator information
18-APR-2008	Response to FDA comments
18-APR-2008	Submission of new investigator information
25-APR-2008	Pre-BLA meeting
29-APR-2008	FDA request for teleconference
30-APR-2008	Response to FDA request regarding changes to protocol
30-APR-2008	Submission of SN 061
08-MAY-2008	Submission of new investigator information
13-MAY-2008	Submission of protocol amendment and revised protocol
13-MAY-2008	Submission of new protocol and new investigator information Transfer of obligation
15-MAY-2008	Addendum to investigator brochure, version 10
29-MAY-2008	Responses to FDA comments
30-MAY-2008	Submission of protocol amendment and revised protocol
06-JUN-2008	Response to 6/5/08 FDA request regarding amount of site activity and status
09-JUN-2008	Submission of new investigator information
26-JUN-2008	Submission of annual report

Date	Event
01-JUL-2008	Submission of documents for 7/9/08 teleconference
09-JUL-2008	Teleconference with FDA
04-AUG-2008	Submission of protocol amendment
07-AUG-2008	Submission of new investigator information
21-AUG-2008	Submission of prostate meeting information
26-AUG-2008	Submission of new investigator information
27-AUG-2008	Response to FDA request to provide information for 7/9/2008 teleconference
28-AUG-2008	Submission of CMC-DMF amendment
28-AUG-2008	Submission of investigator brochure, version 11
04-SEP-2008	Submission of new investigator information
10-SEP-2008	Submission of protocol amendment
19-SEP-2008	Discussion on prostate meeting with FDA
25-SEP-2008	Submission of protocol amendment and revised protocol
30-SEP-2008	Response to FDA request for revisions to informed consent form
01-OCT-2008	Submission of new investigator information
07-OCT-2008	Submission of protocol amendment
08-OCT-2008	Submission of protocol amendment and revised protocol
17-OCT-2008	Submission of new investigator information
17-OCT-2008	Request for SPA
23-OCT-2008	Submission of request for review of proposed trade name
28-OCT-2008	Discussion regarding SPA request, safety issues, and DMC
29-OCT-2008	Submission of new investigator information
30-OCT-2008	Submission of final clinical study report
07-NOV-2008	Submission of amendment to SPA
07-NOV-2008	Submission of revised case report form
18-NOV-2008	Submission of clinical report protocol
19-NOV-2008	Submission of new investigator information
19-NOV-2008	Submission of slides for 11/20/08 teleconference
18-DEC-2008	Submission of new investigator information
19-DEC-2008	FDA questions concerning revisions to study
06-JAN-2009	Submission of new investigator information

Date	Event
22-JAN-2009	Submission of addendum to investigator brochure, version 11
27-JAN-2009	Request for FDA assistance on SPA
30-JAN-2009	Submission of new investigator information
18-FEB-2009	Email correspondence with FDA regarding DMC
19-FEB-2009	Submission of new investigator information
24-FEB-2009	Submission of new protocol, protocol amendment, new investigator information, and transfer of obligation
25-FEB-2009	Request for SPA
26-FEB-2009	Submission of protocol amendments and revised protocols
03-MAR-2009	Letter from FDA regarding SPA comments
17-MAR-2009	Response to FDA regarding SPA comments
20-MAR-2009	Request for type C meeting
20-MAR-2009	Meeting request regarding SN694
23-MAR-2009	Response to FDA request for additional information
26-MAR-2009	Submission of archival copy of approved protocols and amendments approved under SPA
27-MAR-2009	FDA response regarding logistics for type C meeting
01-APR-2009	Submission of new investigator information
03-APR-2009	Correspondence with FDA regarding converting to eCTD
06-APR-2009	Submission of summary of changes, revised protocol, protocol amendments, abbreviated statistical analysis plan, IRC charter and data monitoring committee information
06-APR-2009	Submission of change in investigator information
22-APR-2009	Submission of protocol amendment and revised protocol
30-APR-2009	Submission of background document for type C meeting
30-APR-2009	Safety teleconference with FDA
01-MAY-2009	Teleconference regarding Type C questions
27-MAY-2009	Submission of protocol amendment and revised protocol
28-MAY-2009	Submission of changes in investigator information
28-MAY-2009	FDA comments regarding upcoming teleconference
03-JUN-2009	Teleconference with FDA regarding comparability process
11-JUN-2009	Submission of protocol amendment and revised protocol
25-JUN-2009	Submission of annual report
14-JUN-2009	Submission of change in investigator information

Date	Event
14-JUL-2009	Submission of change in investigator information
28-JUL-2009	Response to FDA request for meeting minutes from teleconference
07-AUG-2009	Submission of amendment to CMC information by reference to DMF amendment
07-AUG-2009	Submission of new protocol, protocol amendment, revised protocol, and new investigator information
24-AUG-2009	Submission of investigator brochure, version 12
25-AUG-2009	Submission of administrative letter
14-SEP-2009	Request for review of proposed tradename
29-SEP-2009	Submission of addendum to investigator brochure, version 12
14-OCT-2009	Submission of protocol amendment, revised protocol, administrative letter
23-OCT-2009	Submission of new investigator information and change in investigators
06-NOV-2009	Submission of protocol amendment and revised protocol
13-NOV-2009	Submission of preliminary data
03-DEC-2009	Request for type C meeting
07-DEC-2009	Purchase of MDS Pharma Services's central lab by Clearstone Central Laboratories
09-DEC-2009	Submission of change in investigator information
11-DEC-2009	Submission of background document for type C meeting
14-DEC-2009	Request for type B pre-BLA meeting
12-JAN-2010	Submission of administrative letters and protocol amendment
19-JAN-2010	Request for waiver of requirements regarding supportive documentation
25-JAN-2010	Submission of clarification questions to FDA regarding BLA
02-FEB-2010	Correspondence with FDA regarding topics for background document discussion on 3/4/2010
22-FEB-2010	Submission of new investigator information and change of investigator information
02-MAR-2010	FDA comments on ipilimumab pre-BLA
12-MAR-2010	Request for type C meeting
23-MAR-2010	Submission of protocol amendment and revised protocol
23-MAR-2010	FDA grant of type C meeting request
24-MAR-2010	Correspondence with FDA regarding informal meeting request with CDRH
24-MAR-2010	FDA grant of waiver for studies not conducted under IND
09-APR-2010	Request for proprietary name review
26-APR-2010	Submission of comparability type C meeting background document

Date	Event
05-MAY-2010	Submission of clinical study report
05-MAY-2010	FDA request for information on Al monkey toxicity
07-MAY-2010	Response to FDA request for information regarding patients with brain metastases
18-MAY-2010	Response to FDA request for information regarding pre-license inspections
16-JUN-2010	Response to FDA comments on enhanced pre- and post-natal development protocol in monkeys
22-JUN-2010	Submission of annual report
25-JUN-2010	Submission of initial BLA application for ipilimumab injection (5mg/1mL)
07-JUL-2010	Submission of administrative letter, revised protocol, and new investigator information
08-JUL-2010	FDA acknowledgement of BLA receipt
12-JUL-2010	BLA monthly update teleconference
14-JUL-2010	Submission regarding expanded access program
02-AUG-2010	Response to FDA request for information regarding statistics
10-AUG-2010	Submission of proposal for providing high-level OS
13-AUG-2010	Response to FDA regarding annotated package insert
16-AUG-2010	Response to FDA questions
16-SEP-2010	Response to FDA regarding protocol and SPA
20-SEP-2010	Response to FDA nonclinical requests
28-SEP-2010	Response to FDA requests regarding ECGs
28-SEP-2010	FDA grant of tradename YERVOY
30-SEP-2010	Response to FDA request to provide study
04-OCT-2010	Submission of investigator brochure, version 13
19-OCT-2010	Response to FDA questions
12-NOV-2010	Submission of administrative letter, protocol amendment, and new investigator information
16-NOV-2010	Response to FDA requests from 11/4/2010
02-DEC-2010	Submission of statistical analysis plan
13-DEC-2010	Response to FDA's request for information regarding safety review
06-JAN-2011	Response to FDA package insert revisions
12-JAN-2011	Response to FDA request for CMC information
18-JAN-2011	Response to FDA request regarding post marketing requirement request
20-JAN-2011	Response to FDA request regarding proposed REMS comments

Date	Event
07-FEB-2011	Response to FDA request regarding immunogenicity
08-FEB-2011	Response to FDA request regarding proposed labeling and cases for further examination
08-FEB-2011	Response to FDA request regarding business card in packaging
22-FEB-2011	Response to FDA request for additional CMC information
24-FEB-2011	Response to FDA request regarding package insert revisions
01-MAR-2011	Response to FDA request regarding post-marketing requirements and medication guide
01-MAR-2011	Response to FDA request for CMS and PMC information
02-MAR-2011	Response to FDA request regarding postmarketing commitments
11-MAR-2011	Response to FDA request regarding carton/container labels
14-MAR-2011	Response to FDA request regarding package insert revisions
14-MAR-2011	Response to FDA request regarding REMS and supporting document assessment revisions
14-MAR-2011	Response to FDA request regarding postmarketing commitments
15-MAR-2011	Submission of revised version of REMS materials
24-MAR-2011	Response to FDA package insert revisions and REMS material
25-MAR-2011	Response to FDA revisions to BMS REMS webpage
25-MAR-2011	Approval of BLA for YERVOY (ipilimumab)
01-APR-2011	Submission of final product label/structured product label management guide submission