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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	835

Application Number	Patent No.: 7,097,840
Filing Date	Issued: August 29, 2006
First Named Inventor	Sharon ERICKSON
Art Unit	1643
Examiner Name	H. Sang
Attorney Docket Number	146392019700

ENCLOSURES (*Check all that apply*)

- [x] Fee Transmittal Form + 4 copies (5 pages)
- [ ] Fee Attached
- [ ] Amendment/Reply
- [ ] After Final
- [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
- [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

See Remarks

Remarks

- Application for Extension of Patent Term under 35 U.S.C. § 156 + 4 copies (80 pages)
- Attachment A + 4 copies (15 pages)
- Attachment B + 4 copies (115 pages)
- Attachment C + 4 copies (70 pages)
- Attachment D + 4 copies (475 pages)
- Attachment E + 4 copies (10 pages)
- Attachment F + 4 copies (10 pages)
- Attachment G + 4 copies (15 pages)
- Attachment H + 4 copies (20 pages)
- Attachment I + 4 copies (15 pages)
- Return Receipt Postcard

RECEIVED
APR 17 2013
PATENT EXTENSION
OPLA

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MORRISON & FOERSTER LLP (Customer No. 25226)		
Signature	Catherine M. Polizzi		
Printed name	Catherine M. Polizzi		
Date	April 17, 2013	Reg. No.	40,130

I hereby certify that this paper is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EM 295528087 US, on the date shown below in an envelope addressed to: Mail Stop Hatch-Waxman PTE
Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: April 17, 2013 Signature: _____ (Shannon Reaney)

pa-1583991

PTO/SB/17 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL

Complete if known	
Application Number	Patent No.: 7,097,840
Filing Date	Issued: August 29, 2006
First Named Inventor	Sharon ERICKSON
Examiner Name	H. Sang
Art Unit	1643
Practitioner Docket No.	146392019700

☐ Applicant asserts small entity status. See 37 CFR 1.27

☐ Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously

TOTAL AMOUNT OF PAYMENT ($) 1,120.00

METHOD OF PAYMENT (check all that apply)

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

☒ Deposit Account Deposit Account Number: 03-1952 Deposit Account Name: Morrison & Foerster 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 underpayment of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayment of fee(s)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

	FILING FEES			SEARCH FEES			EXAMINATION FEES			
Application Type	U ($)	S ($)	M ($)	U ($)	S ($)	M ($)	U ($)	S ($)	M ($)	Fees Paid ($)
Utility	280	140*	70	600	300	150	720	360	180	
Design	180	90	45	120	60	30	460	230	115	
Plant	180	90	45	380	190	95	580	290	145	
Reissue	280	140	70	600	300	150	2,160	1,080	540	
Provisional	260	130	65	0	0	0	0	0	0	

* The $140 small entity status filing fee for a utility application is further reduced to $70 for a small entity status applicant who files the application via EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	Undiscounted Fee ($)	Small Entity Fee ($)	Micro Entity Fee ($)
Each claim over 20 (including Reissues)	80	40	20
Each independent claim over 3 (including Reissues)	420	210	105
Multiple dependent claims	780	390	195

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

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

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

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 $400 ($200 for small entity) ($100 for micro 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 _____ - 100 = _____ Extra Sheets _____ /50 = _____ Number of each additional 50 or fraction thereof (round up to a whole number) x Fee ($) _____ = Fee Paid ($) _____

4. OTHER FEE(S) Fees Paid ($)

Non-English specification, $130 fee (no small or micro entity discount) _____

Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, $400 fee ($200 small or micro entity) _____

Other (e.g., late filing surcharge): 1457 Extension of term of patent 1,120.00

SUBMITTED BY					
Signature	Catherine M. Polizzi	Registration No. (Attorney/Agent)	40,130	Telephone	(650) 813-5651
Name (Print/Type)	Catherine M. Polizzi			Date	April 17, 2013

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Dated: April 17, 2013 Signature: _____ (Shannon Reaney)

pa-1583973

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Dated: April 17, 2013 Signature: (Shannon Reaney)

Docket No.: 146392019700
Client Ref. No.: 10813

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Sharon Erickson *et al.*

Patent No.: 7,097,840

Issued: August 29, 2006

Application No: 09/811,123

For: METHODS OF TREATMENT USING ANTI-ERBB ANTIBODY-MAYTANSINOID CONJUGATES – Application for § 156 Patent Term Extension

Attorney Docket No: 146392019700

Assignees: Genentech, Inc. and ImmunoGen, Inc.

Unit: Office of Patent Legal Administration

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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

Dear Madam:

Applicant, Genentech, Inc., hereby submits this application for extension of the term of United States Letters Patent No. 7,097,840 under 35 U.S.C. § 156 by providing the following information in accordance with the requirements specified in 37 C.F.R. § 1.740.

A statement executed by an authorized representative of co-owner ImmunoGen, Inc. attesting that co-owner Genentech, Inc. is authorized to act as an agent of ImmunoGen, Inc. for extension of the term of United States Letters Patent No. 7,097,840 under 35 U.S.C. § 156 is included as Attachment A.

12/03/2013 CKHLOK 00000009 031952 09811123
01 FC:1457 1120.00 DA

pa-1580256

Applicant represents that co-owners Genentech, Inc. and ImmunoGen, Inc. are the assignees of the entire interest in and to United States Letters Patent No. 7,097,840 granted to Sharon Erickson, Ralph Schwall, Mark Sliwkowski, and Walter Blattler (Erickson *et al.*) by virtue of an assignment of such patent from Sharon Erickson , Ralph Schwall, and Mark Sliwkowski to Genentech, Inc., recorded July 19, 2001, at Reel 011977, Frame 0409 and by virtue of assignments of such patent from Walter Blattler to ImmunoGen, Inc., recorded December 20, 2002, at Reel 013640, Frame 0657 and on April 4, 2003, at Reel 013566, Frame 0732.[1]

1. Identification of the Approved Product [§ 1.740(a)(1)]

The name of the approved product is KADCYLA™. The name of the active ingredient of KADCYLA™ is ado-trastuzumab emtansine. Ado-trastuzumab emtansine has also been referred to as trastuzumab emtansine. Applicant uses the nomenclature ado-trastuzumab emtansine, which is the same nomenclature used in the product label for KADCYLA™. Ado-trastuzumab emtansine is a HER2-targeted antibody-drug conjugate which contains the humanized anti-HER2 IgG1, trastuzumab, covalently linked to the microtubule inhibitory drug DM1 (the maytansine derivative $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine) via the stable thioether linker MCC (4-[N-maleimidomethyl] cyclohexane-1-carboxylate). *See* Description section of product label, provided as Attachment B.

2. Federal Statute Governing Regulatory Approval of the Approved Product [§ 1.740(a)(2)]

The approved product was subject to regulatory review under, *inter alia*, the Public Health Service Act (42 U.S.C. § 201 *et seq.*) and the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355 *et seq.*).

3. Date of Approval for Commercial Marketing [§ 1.740(a)(3)]

KADCYLA™ was approved for commercial marketing or use under § 351 of the Public Health Service Act on February 22, 2013.

4. Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product [§ 1.740(a)(4)]

(a) The name of the active ingredient of KADCYLA™ is ado-trastuzumab emtansine. Ado-trastuzumab emtansine is a HER2-targeted antibody-drug conjugate which contains the humanized anti-HER2 IgG1, trastuzumab, covalently linked to the microtubule inhibitory drug DM1 (the maytansine derivative $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine) via the stable thioether linker MCC (4-[N-maleimidomethyl] cyclohexane-1-carboxylate). *See* Description section of product label, provided as Attachment B.

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[1] Additionally, a change of address of ImmunoGen, Inc. was recorded March 2, 2010, at Reel 024006, Frame 0734.

(b) Applicant certifies that ado-trastuzumab emtansine had not been 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 prior to the approval granted on February 22, 2013 to the present Applicant.

(c) Ado-trastuzumab emtansine has been approved, as a single agent, for the treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination. Patients should have either: received prior therapy for metastatic disease, or developed disease recurrence during or within six months of completing adjuvant therapy. *See* Indications and Usage section of ado-trastuzumab emtansine product label, provided as Attachment B.

(d) Ado-trastuzumab emtansine was approved for commercial marketing pursuant to § 351 of the Public Health Service Act (42 U.S.C. § 262) under Genentech's existing Department of Health and Human Services (DHHS) U.S. License No. 1048. *See* ado-trastuzumab emtansine approval letter, provided as Attachment C.

5. Statement Regarding Timeliness of Submission of Patent Term Extension Request [§ 1.740(a)(5)]

Applicant certifies that this application for patent term extension is being timely submitted within the sixty (60) day period permitted for submission specified in 35 U.S.C. § 156(d)(1) and 37 C.F.R. § 1.720(f). The last date on which this application can be submitted is April 22, 2013.

6. Complete Identification of the Patent for Which Extension Is Being Sought [§ 1.740(a)(6)]

The complete identification of the patent for which an extension is being sought is as follows:

(a)	Names of the inventors:	Sharon Erickson, Ralph Schwall, Mark Sliwkowski, and Walter Blattler
(b)	Patent Number:	7,097,840 ("the '840 patent")
(c)	Date of Issue:	August 29, 2006
(d)	Date of Expiration:	January 27, 2023 (20 years from March 16, 2001 plus 682 days Patent Term Adjustment)[2]

[2] The '840 patent received 682 days of Patent Term Adjustment. *See* the '840 patent provided as Attachment D.

7. Copy of the Patent for Which an Extension Is Being Sought [§ 1.740(a)(7)]

A copy of the '840 patent is provided as Attachment D to the present application.

8. Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payment, or Reexamination Certificate [§ 1.740(a)(8)]

(a) The '840 patent is not subject to a Terminal Disclaimer.

(b) A copy of a Certificate of Correction issued with respect to the '840 patent on March 20, 2007 is provided in Attachment E.[3]

(c) The '840 patent issued on August 29, 2006. The first maintenance fee was paid on January 29, 2010 (*See* Attachment F). The window for paying the second maintenance fee opens August 29, 2013 (*See* Attachment F). Therefore, no maintenance fee is currently due for the '840 patent.

(d) The '840 patent has not been the subject of a reexamination proceeding and, thus, no re-examination certificate has been issued.

9. Statement Regarding Patent Claims Relative to Approved Product [§ 1.740(a)(9)]

The statements below are made solely to comply with the requirements of 37 C.F.R. § 1.740(a)(9). Applicant notes that, as the M.P.E.P. acknowledges, § 1.740(a)(9) does not require an applicant to show whether or how the listed claims would be infringed, and that this question cannot be answered without specific knowledge concerning acts performed by third parties. As such, these comments are not an assertion or an admission of Applicant as to the scope of the listed claims, or whether or how any of the listed claims would be infringed, literally or under the doctrine of equivalents, by the manufacture, use, sale, offer for sale or the importation of any product.

(a) At least claims 1-3, 6-17, 20-26, 28-29 and 42-44 claim the active pharmaceutical ingredient in the approved product or the approved product or a method that may be used to make or use that ingredient or product.

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[3] The Patent and Trademark Office (hereafter "the Office") issued a Certificate of Correction for the '840 patent on December 12, 2006. On December 13, 2006, the Office sent Patent Owners a letter noting that the Certificate of Correction issued on December 12, 2006 listed the incorrect issue date for the patent and was therefore issued in error. A Certificate of Correction was issued January 9, 2007, which listed the correct issue date and superseded the December 12, 2006 Certificate of Correction. A further Certificate of Correction issued March 20, 2007, superseding both the December 12, 2006 and January 9, 2007 Certificates of Correction.

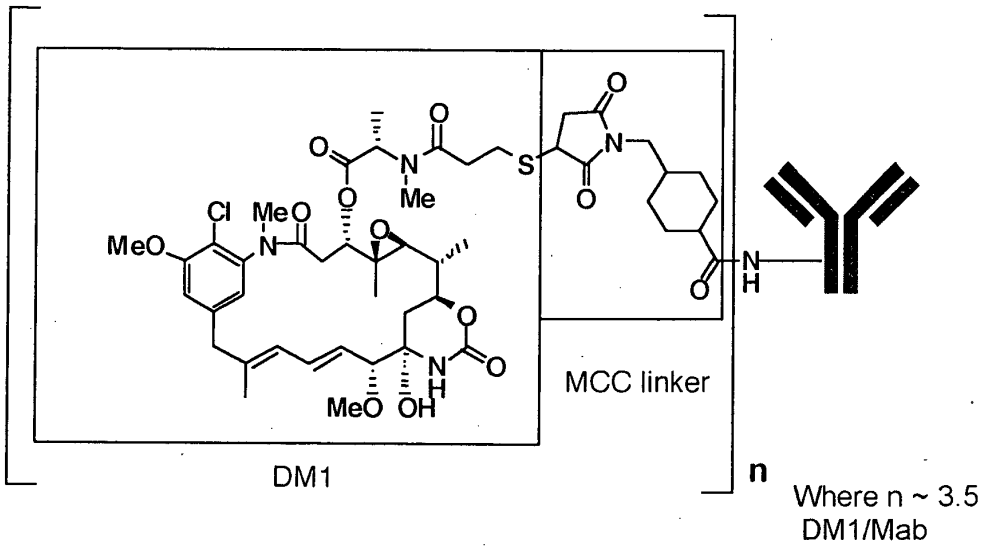
(b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows how at least one of the above-listed claims of the '840 patent claim the approved product.

(1) Description of the approved product

The name of the approved product is KADCYLA™. The name of the active ingredient of KADCYLA™ is ado-trastuzumab emtansine. Ado-trastuzumab emtansine is a HER2-targeted antibody-drug conjugate which contains the humanized anti-HER2 IgG1, trastuzumab, covalently linked to the microtubule inhibitory drug DM1 (the maytansine derivative $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine) via the stable thioether linker MCC (4-[N-maleimidomethyl] cyclohexane-1-carboxylate). *See* Description section of product label, provided as Attachment B.

The antibody trastuzumab, is a well characterized recombinant monoclonal antibody product produced by mammalian (Chinese hamster ovary) cells, and the small molecule components (DM1 and MCC) are produced by chemical synthesis. Ado-trastuzumab emtansine contains an average of 3.5 DM1 molecules per antibody. *Id.*

Ado-trastuzumab emtansine has the following chemical structure:



The bracketed structure is DM1 plus MCC which represents the emtansine component. The n is, on average, 3.5 DM1 molecules per trastuzumab (Mab) molecule. *Id.*

(2) Explanation regarding claim 1 of the '840 patent relative to ado-trastuzumab emtansine

Claim 1 of the '840 patent reads:

"A method for the treatment of a tumor in a mammal, comprising the steps of (i) identifying said tumor as being characterized by overexpression of an ErbB2 receptor and as being a tumor that does not respond, or responds poorly, to treatment with an anti-ErbB2 antibody which binds to the 4D5 epitope and which has a growth inhibitory effect on SK-BR-3 cells, and (ii) administering to a mammal having said tumor a therapeutically effective amount of a conjugate of an anti-ErbB2 antibody which binds to the 4D5 epitope with a maytansinoid."

KADCYLA™ is indicated, as a single agent, for the treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination (*see* Indications and Usage section of ado-trastuzumab emtansine product label, Attachment B). The approved use is thus for the treatment of a tumor in a mammal.

Detection of HER2 protein overexpression or gene amplification is necessary for selection of patients appropriate for KADCYLA™ therapy (*see* HER2 Testing section of ado-trastuzumab emtansine product label, Attachment B). HER2 is synonymous with "ErbB2." *See* the '840 patent, Attachment D, column 6, lines 54-56. The approved use thus requires identifying the tumor as being characterized by overexpression of an ErbB2 receptor.

The approved use of KADCYLA™ is for patients who previously received trastuzumab and a taxane, separately or in combination (*see* Indications and Usage section of ado-trastuzumab emtansine product label, Attachment B). In addition, patients should have either: received prior therapy for metastatic disease, or developed disease recurrence during or within six months of completing adjuvant therapy. *Id.*

Trastuzumab is an anti-ErbB2 antibody also known by its trade name HERCEPTIN® (*See* the '840 patent, Attachment D, column 7, lines 1-8).[4] HERCEPTIN® is an anti-ErbB2 antibody which binds to the 4D5 epitope and which has a growth inhibitory effect on SK-BR-3 cells (*see* the '840 patent, Attachment D, column 2, lines 44-52; column 3, lines 8-14; column 14, lines 35-46; Example 2 and Figure 6).

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[4] *See also* drug details for HERCEPTIN®, provided in Attachment G, available at the FDA website address:
http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Set_Current_Drug&ApplNo=103792&DrugName=HERCEPTIN&ActiveIngred=TRASTUZUMAB&SponsorApplicant=GENENTECH&ProductMktStatus=1&goto=Search.DrugDetails

Accordingly, the approved use of KADCYLA™ is for the treatment of a patient whose tumor does not respond, or responds poorly, to treatment with an anti-ErbB2 antibody which binds to the 4D5 epitope and which has a growth inhibitory effect on SK-BR-3 cells.

Ado-trastuzumab emtansine is an antibody-drug conjugate which contains the humanized anti-HER2 IgG1, trastuzumab, covalently linked to the microtubule inhibitory drug DM1 (the maytansine derivative $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine) via the stable thioether linker MCC (4-[N-maleimidomethyl] cyclohexane-1-carboxylate) (*see* Description section of ado-trastuzumab emtansine product label, Attachment B). As stated above, trastuzumab is an anti-ErbB2 antibody which binds to the 4D5 epitope. DM1 is a maytansinoid. *Id.* KADCYLA™ is indicated, as a single agent, for the treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination (*see* Indications and Usage section of ado-trastuzumab emtansine product label, Attachment B). Ado-trastuzumab emtansine is thus a conjugate of an anti-ErbB2 antibody which binds to the 4D5 epitope with a maytansinoid and is approved to be administered in a therapeutically effective amount to treat breast cancer.

The approved use of ado-trastuzumab emtansine thus meets the limitations of claim 1.

10. Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [§ 1.740(a)(10)]

(a) Patent Issue Date

The '840 patent was issued on August 29, 2006.

(b) IND Effective Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(A)]

The date that an exemption under § 505(i) of the Federal Food, Drug and Cosmetic Act became effective (*i.e.*, the date that an investigational new drug application ("IND") became effective) for ado-trastuzumab emtansine was January 18, 2006.[5] The IND was assigned number 071072.

(c) BLA Submission Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(B)]

The BLA was submitted by Genentech to the FDA on August 24, 2012. The BLA was assigned number 125427. A copy of the letter from the FDA acknowledging receipt of the BLA and reflecting the BLA submission date is provided in Attachment H.

(d) BLA Issue Date [35 U.S.C. § 156(g)(1)(B)(ii); 37 C.F.R. § 1.740(a)(10)(i)(C)]

The FDA approved BLA 125427, authorizing the marketing of ado-trastuzumab emtansine, on February 22, 2013. Ado-trastuzumab emtansine was approved under Department of Health and Human Services (DHHS) U.S. License No. 1048. A copy of the approval letter from the FDA is provided as Attachment C.

[5] 21 C.F.R. § 312.40(b)(1). The IND was submitted to the FDA on December 16, 2005 and was received by the FDA on December 19, 2005. The IND became effective on January 18, 2006, 30 days after receipt of the IND by the FDA.

11. Summary of Significant Events During Regulatory Review Period [§ 1.740(a)(11)]

Pursuant to 37 C.F.R. § 1.740(a)(11), the following provides a brief description of the activities of Genentech, Inc. before the FDA in relation to the regulatory review of ado-trastuzumab emtansine. The brief description lists significant events that occurred during the regulatory review period for the approved product. In several instances, communications to or from the FDA are referenced. Pursuant to 37 C.F.R. § 1.740(a)(11), 21 C.F.R. § 60.20(a), and M.P.E.P. § 2753, copies of all such communications are not provided in this application, but can be obtained from records maintained by the FDA.

- On December 16, 2005, Genentech submitted to the FDA an Investigational New Drug (IND) application for ado-trastuzumab emtansine. The FDA assigned the IND application number 071072.

- On December 16, 2005, Genentech submitted new protocol TDM3569g for the first human clinical trial (Phase I). The protocol indicated that the study would not be initiated until an IND covering the clinical trial came into effect.

- The Phase I clinical trial was initiated in April of 2006 followed by Phase II and Phase III clinical trials.

- On May 7, 2007, Genentech submitted new protocol TDM4258g.

- On November 2, 2007, Genentech submitted new protocol TDM4374g.

- On May 6, 2008, Genentech submitted new protocol TDM4450g, a revised protocol for TDM4374g, and a change in protocol TDM4258g, Amendment 1.

- On August 8, 2008, representatives of Genentech and the FDA participated in an End-of-Phase II meeting to discuss and agree on the design of the pivotal Phase III trial.

- On October 15, 2008, Genentech submitted new protocol TDM4370g, a phase III international, multi-center, open-label, randomized study of ado-trastuzumab emtansine versus lapatinib plus capecitabine, in patients with HER2-positive unresectable locally advanced breast cancer (LABC) or metastatic breast cancer (MBC) who have progression of disease after receiving trastuzumab and a taxane.

- On May 15, 2009, Genentech submitted new protocol TDM4688g.

- On March 15, 2012 and May 30, 2012 representatives of Genentech and the FDA participated in Type C and Type B pre-BLA submission meetings, respectively, to discuss information requirements for the BLA and the acceptability of the Phase III trial results to serve as the basis for the BLA.

- On August 24, 2012, Genentech submitted a BLA for ado-trastuzumab emtansine as a single agent for the treatment of patients with human epidermal growth factor

2 (HER2)-positive unresectable, locally advanced or metastatic breast cancer who have received prior treatment with trastuzumab and a taxane.

- FDA acknowledged receipt of the BLA for ado-trastuzumab emtansine via a communication mailed to Genentech dated October 23, 2012. The letter indicated that FDA had assigned the Submission Tracking Number (STN) of BLA 125427 to the BLA (*see* Attachment H).

- On February 22, 2013, FDA approved BLA 125427, issuing marketing authorization for ado-trastuzumab emtansine (*see* Attachment C).

12. Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R. § 1.740(a)(12)]

(a) In the opinion of the Applicant, the '840 patent is eligible for an extension under § 156 because:

(i) one or more claims of the '840 patent claim the approved product or a method of making or using the approved product (35 U.S.C. § 156(a));

(ii) the '840 patent has not expired before submission of this application (35 U.S.C. § 156(a)(1));

(iii) the term of the '840 patent has not been previously extended on the basis of § 156 (35 U.S.C. § 156(a)(2));

(iv) the application for extension is submitted by an owner of record or an agent authorized to act on behalf of the owner of record in accordance with the requirements of paragraphs (1) through (4) of 35 U.S.C. § 156(d) and the rules of the Patent and Trademark Office (35 U.S.C. § 156(a)(3));

(v) the product, KADCYLA™, has been subject to a regulatory review period before its commercial marketing or use (35 U.S.C. § 156(a)(4));

(vi) the commercial marketing or use of the product, KADCYLA™, after the regulatory review period is the first permitted commercial marketing or use of the product under the provisions under the Public Health Service Act, Section 351, under which such regulatory review occurred (35 U.S.C. § 156(a)(5)(A));

(vii) no other patent has been extended pursuant to § 156 on the basis of the regulatory review process associated with the approved product (35 U.S.C. § 156(c)(4));

(viii) the applicant for marketing approval exercised due diligence within the meaning of § 156(d)(3) during the period of regulatory review;

(ix) the present application is being submitted within the 60-day period following the approval date of the approved product, pursuant to § 156(d); and

(x) this application otherwise complies with all requirements of 35 U.S.C. § 156 and applicable rules and procedures.

(b) The period by which the term of the '840 patent is requested by Applicant to be extended is **1,277 days** (35 U.S.C. § 156(c)).

(c) The requested period of extension of term for the '840 patent corresponds to the regulatory review period that is eligible for extension pursuant to § 156, based on the facts and circumstances of the regulatory review associated with the approved product and the issuance of the '840 patent. The period was determined as follows.

(i) The relevant dates for calculating the regulatory review period, based on the events discussed in the section above, are the following:

Exemption under FDCA § 505(i) became effective	January 18, 2006
Patent was granted	August 29, 2006
Biologics License Application (BLA) under PHSA § 351 was submitted	August 24, 2012
BLA was approved	February 22, 2013

(ii) The '840 patent was granted during the period specified in § 156(g)(1)(B)(i) (the period of 2,411 days calculated from the date of the grant of the exemption under § 505(i) of the FDCA (January 18, 2006) until the date of submission of the BLA (August 24, 2012)). Pursuant to §§ 156(c), the calculated regulatory review period therefore includes a component of time between when the patent was granted (August 29, 2006) and when the BLA was submitted (August 24, 2012) (1/2 of 2,187 days or 1,094 days).[6]

(iii) The '840 patent was granted prior to the start of the period specified in §§ 156(g)(1)(B)(ii) (the period from the date of submission of the BLA until the date of BLA approval). The number of days which the applicant did not act with due diligence is zero (0) days. The regulatory review period under § 156(c) therefore includes a component of time between when the BLA was submitted and when the BLA was approved (183 days).

(iv) The period determined according to §§ 156(c) and (g)(1) for the approved product is 1,277 days.

(v) The '840 patent will expire on January 27, 2023.

(vi) The date of approval of the approved product is February 22, 2013.

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[6] Under 37 C.F.R. § 1.775(d)(1)(iii), half days are ignored for purposes of subtraction.

(vii) The date that is fourteen years from the date of approval of the approved product is February 22, 2027.

(viii) The date that is five years from the expiration date of the '840 patent is January 27, 2028.

(ix) The date that is provided by adding the number of days determined according to §§ 156(c) and (g)(1) for the approved product (1,277 days) to the expiration date of the '840 patent is July 27, 2026.

(x) The date that is fourteen years from the date of approval of the approved product (February 22, 2027) is later than the date that is provided by adding the number of days determined according to §§ 156(c) and (g)(1) for the approved product to the expiration date of the '840 patent (July 27, 2026). As such, the period by which the patent may be extended is not limited by the fourteen-year rule of §156(c)(3).

(xi) The date that is five years from the expiration date of the '840 patent (January 27, 2028) is later than the date that is provided by adding the number of days determined according to §§ 156(c) and (g)(1) for the approved product to the expiration date of the '840 patent (July 27, 2026). As such, the period by which the patent may be extended is not limited by the five-year rule of §156(g)(6)(a).

(xii) The '840 patent issued after the effective date of Public Law No. 98-417. As such, the two- or three-year limit of 35 U.S.C. § 156(g)(6)(C) does not apply.

13. Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Pursuant to 37 C.F.R. § 1.740(a)(13), Applicant acknowledges its duty to disclose to the Director of the PTO and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, particularly as that duty is defined in 37 C.F.R. § 1.765.

14. Applicable Fee [§ 1.740(a)(14)]

Payment of the fee prescribed in 37 C.F.R. § 1.20(j) for a patent term extension application under 35 U.S.C. § 156 is authorized to be charged against deposit account no. 03-1952 referencing docket number 146392019700. The undersigned also authorizes any additional required fees to be deducted from, or any overpayments to be credited to, deposit account no. 03-1952.

15. Name and Address for Correspondence [§ 1.740(a)(15)]

Please direct all inquiries, questions, and communications regarding this application for term extension to:

Catherine M. Polizzi
Registration No.: 40,130
MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Phone: 650/813-5651
Facsimile: 650/494-0792

The correspondence address for U.S. Patent No. 7,097,840 is unchanged for all other purposes. A Power of Attorney granted to the Practitioners associated with Customer Number 25226 by Genentech, Inc. (*see* Attachment I) and ImmunoGen, Inc. (*see* Attachment A) are provided.

Two additional copies of this application are enclosed, in compliance with 37 C.F.R. § 1.740(b). Applicant also provides herewith two further copies of the application for the convenience of the Office, pursuant to M.P.E.P. § 2753.

Sincerely,

By: Catherine M. Polizzi

Catherine M. Polizzi
Registration No.: 40,130
MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Phone: 650/813-5651
Facsimile: 650/494-0792

Dated: April 17, 2013

INDEX OF ATTACHMENTS

Attachment A

Statement of ImmunoGen, Inc. attesting that Genentech, Inc. is authorized to act as an agent of ImmunoGen, Inc. for extension of the term of United States Letters Patent 7,097,840 under 35 U.S.C. § 156 and conferring Power of Attorney to Practitioners

Docket No.: 146392019700
Client Ref. No.: 10813

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Sharon Erickson *et al.*

Patent No.: 7,097,840

Issued: August 29, 2006

Application No: 09/811,123

For: METHODS OF TREATMENT USING ANTI-ERBB ANTIBODY-MAYTANSINOID CONJUGATES – Application for § 156 Patent Term Extension

Attorney Docket No: 146392019700

Assignees: Genentech, Inc. and ImmunoGen, Inc.

Unit: Office of Patent Legal Administration

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AUTHORIZATION AND POWER OF ATTORNEY TO FILE APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

As an authorized representative of ImmunoGen, Inc., co-owner of the entire right, title and interest in U.S. Patent No. 7,097,840 (the "'840 Patent"), I hereby authorize Genentech, Inc., co-owner and exclusive licensee of the '840 Patent, to act as agent for ImmunoGen, Inc., in the submission of a patent term extension application under 35 U.S.C. § 156 for the '840 Patent. I understand that counsel for Genentech, Morrison & Foerster LLP, will file and prosecute this patent term extension application and hereby grant Morrison & Foerster LLP any authorization from ImmunoGen, Inc., necessary for Morrison & Foerster LLP to act in this capacity. In that regard, practitioners associated with Customer Number 25226 are appointed to file and prosecute the patent term extension application for the '840 Patent and to transact all business in the United States Patent and Trademark Office connected with this patent term extension

application. Please direct all correspondence regarding this patent term extension application to Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, CA 94304-1018. The correspondence address for the '840 Patent is to be unchanged for all other purposes.

Date 4/16/2013

Respectfully submitted,

By _____

Name: Craig Barrows

Title: General Counsel

Phone: (781) 895-0600

pa-1582245

Attachment B

Ado-trastuzumab emtansine Product Label

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use KADCYLA safely and effectively. See full prescribing information for KADCYLA.

KADCYLA™ (ado-trastuzumab emtansine) for injection, for intravenous use
Initial U.S. Approval: *2013*

WARNING: HEPATOTOXICITY, CARDIAC TOXICITY, EMBRYO-FETAL TOXICITY

See full prescribing information for complete boxed warning

- **Do not substitute KADCYLA for or with trastuzumab. (2.1)**
- **Hepatotoxicity, liver failure and death have occurred in KADCYLA-treated patients. Monitor hepatic function prior to initiation and prior to each dose. Institute dose modifications or permanently discontinue as appropriate. (2.2, 5.1)**
- **KADCYLA may lead to reductions in left ventricular ejection fraction (LVEF). Assess LVEF prior to initiation. Monitor and withhold dosing or discontinue as appropriate. (2.2, 5.2)**
- **Can cause fetal harm. Advise women of potential risk to the fetus. (5.3, 8.1, 8.6)**

INDICATIONS AND USAGE

KADCYLA is a HER2-targeted antibody and microtubule inhibitor conjugate indicated, as a single agent, for the treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination. Patients should have either:

- Received prior therapy for metastatic disease, or
- Developed disease recurrence during or within six months of completing adjuvant therapy. (1)

DOSAGE AND ADMINISTRATION

- *For intravenous infusion only.* Do not administer as an intravenous push or bolus. Do not use Dextrose (5%) solution. (2.3)
- The recommended dose of KADCYLA is 3.6 mg/kg given as an intravenous infusion every 3 weeks (21-day cycle) until disease progression or unacceptable toxicity. *Do not administer KADCYLA at doses greater than 3.6 mg/kg. Do not substitute KADCYLA for or with trastuzumab.* (2.1)
- Management of adverse events (infusion-related reactions, hepatotoxicity, left ventricular cardiac dysfunction, thrombocytopenia, pulmonary toxicity or peripheral neuropathy) may require temporary interruption, dose reduction, or treatment discontinuation of KADCYLA. (2.2)

DOSAGE FORMS AND STRENGTHS

Lyophilized powder in single-use vials containing 100 mg per vial or 160 mg per vial. (3)

CONTRAINDICATIONS

None. (4)

WARNINGS AND PRECAUTIONS

- Pulmonary Toxicity: Permanently discontinue KADCYLA in patients diagnosed with interstitial lung disease or pneumonitis. (2.2, 5.4)
- Infusion-Related Reactions, Hypersensitivity Reactions: Monitor for signs and symptoms during and after infusion. If significant infusion-related reactions or hypersensitivity reactions occur, slow or interrupt the infusion and administer appropriate medical therapies. Permanently discontinue KADCYLA for life threatening infusion-related reaction. (2.1, 2.2, 5.5)
- Thrombocytopenia: Monitor platelet counts prior to each KADCYLA dose. Institute dose modifications as appropriate. (2.2, 5.6)
- Neurotoxicity: Monitor for signs or symptoms. Withhold dosing temporarily for patients experiencing Grade 3 or 4 peripheral neuropathy. (2.2, 5.7, 13.2)
- HER2 Testing: Perform using FDA-approved tests by laboratories with demonstrated proficiency. (5.8)

ADVERSE REACTIONS

The most common adverse drug reactions (frequency > 25%) with KADCYLA (n=884 treated patients) were fatigue, nausea, musculoskeletal pain, thrombocytopenia, headache, increased transaminases, and constipation. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Genentech at 1-888-835-2555 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

USE IN SPECIFIC POPULATIONS

- Nursing Mothers: Discontinue nursing or discontinue KADCYLA taking into consideration the importance of the drug to the mother. (8.3)
- Females of Reproductive Potential: Counsel females on pregnancy prevention and planning. Encourage patient participation in the MotHER Pregnancy Registry by contacting 1-800-690-6720). (5.3, 8.1, 8.6)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: *02/2013*

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the Full Prescribing Information are not listed.

FULL PRESCRIBING INFORMATION

Do Not Substitute KADCYLA for or with Trastuzumab

WARNING: HEPATOTOXICITY, CARDIAC TOXICITY, EMBRYO-FETAL TOXICITY

- Hepatotoxicity: Serious hepatotoxicity has been reported, including liver failure and death in patients treated with KADCYLA. Monitor serum transaminases and bilirubin prior to initiation of KADCYLA treatment and prior to each KADCYLA dose. Reduce dose or discontinue KADCYLA as appropriate in cases of increased serum transaminases or total bilirubin. (2.2, 5.1)
- Cardiac Toxicity: KADCYLA administration may lead to reductions in left ventricular ejection fraction (LVEF). Evaluate left ventricular function in all patients prior to and during treatment with KADCYLA. Withhold treatment for clinically significant decrease in left ventricular function. (2.2, 5.2)
- Embryo-Fetal Toxicity: Exposure to KADCYLA can result in embryo-fetal death or birth defects. Advise patients of these risks and the need for effective contraception. (5.3, 8.1, 8.6)

1 INDICATIONS AND USAGE

KADCYLA™, as a single agent, is indicated for the treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination. Patients should have either:

- Received prior therapy for metastatic disease, or
- Developed disease recurrence during or within six months of completing adjuvant therapy.

2 DOSAGE AND ADMINISTRATION

2.1 Recommended Doses and Schedules

The recommended dose of KADCYLA is 3.6 mg/kg given as an intravenous infusion every 3 weeks (21-day cycle) until disease progression or unacceptable toxicity. *Do not administer KADCYLA at doses greater than 3.6 mg/kg. Do not substitute KADCYLA for or with trastuzumab.*

Closely monitor the infusion site for possible subcutaneous infiltration during drug administration *[see Warnings and Precautions (5.9)]*.

First infusion: Administer infusion over 90 minutes. Patients should be observed during the infusion and for at least 90 minutes following the initial dose for fever, chills, or other infusion-related reactions *[see Warnings and Precautions (5.5)]*.

Subsequent infusions: Administer over 30 minutes if prior infusions were well tolerated. Patients should be observed during the infusion and for at least 30 minutes after infusion.

2.2 Dose Modifications

KADCYLA dose should not be re-escalated after a dose reduction is made.

If a planned dose is delayed or missed, it should be administered as soon as possible; do not wait until the next planned cycle. The schedule of administration should be adjusted to maintain a 3-

week interval between doses. The infusion may be administered at the dose and rate the patient tolerated in the most recent infusion.

The infusion rate of KADCYLA should be slowed or interrupted if the patient develops an infusion-related reaction. Permanently discontinue KADCYLA for life-threatening infusion-related reactions *[see Warnings and Precautions (5.5)]*.

Management of increased serum transaminases, hyperbilirubinemia, left ventricular dysfunction, thrombocytopenia, pulmonary toxicity or peripheral neuropathy may require temporary interruption, dose reduction or treatment discontinuation of KADCYLA as per guidelines provided in Tables 1 to 5.

Table 1 Recommended Dose Reduction Schedule for Adverse Events

Dose Reduction Schedule	Dose Level
Starting dose	3.6 mg/kg
First dose reduction	3 mg/kg
Second dose reduction	2.4 mg/kg
Requirement for further dose reduction	Discontinue treatment

Hepatotoxicity [see Warnings and Precautions (5.1)]

A reduction in the dose of KADCYLA is recommended in the case of hepatotoxicity exhibited as increases in serum transaminases and/or hyperbilirubinemia (see Tables 2 and 3).

Table 2 Dose Modification Guidelines for Increased Serum Transaminases (AST/ALT)

Grade 2 (> 2.5 to ≤ 5 × ULN)	Grade 3 (> 5 to ≤ 20 × ULN)	Grade 4 (> 20 × ULN)
Treat at same dose level.	Do not administer KADCYLA until AST/ALT recovers to Grade ≤ 2, and then reduce one dose level.	Permanently discontinue KADCYLA.

ALT = alanine transaminase; AST = aspartate transaminase; ULN = upper limit of normal.

Table 3 Dose Modification Guidelines for Hyperbilirubinemia

Grade 2 (> 1.5 to ≤ 3 × ULN)	Grade 3 (> 3 to ≤ 10 × ULN)	Grade 4 (> 10 × ULN)
Do not administer KADCYLA until total bilirubin recovers to Grade ≤ 1, and then treat at same dose level.	Do not administer KADCYLA until total bilirubin recovers to Grade ≤ 1, and then reduce one dose level.	Permanently discontinue KADCYLA.

Permanently discontinue KADCYLA treatment in patients with serum transaminases > 3 x ULN and concomitant total bilirubin > 2 x ULN.

Permanently discontinue KADCYLA in patients diagnosed with nodular regenerative hyperplasia (NRH).

Left Ventricular Dysfunction [see Warnings and Precautions (5.2)]

Table 4 Dose Modifications for Left Ventricular Dysfunction

Symptomatic CHF	LVEF < 40%	LVEF 40% to ≤ 45% and decrease is ≥ 10% points from baseline	LVEF 40% to ≤ 45% and decrease is < 10% points from baseline	LVEF > 45%
Discontinue KADCYLA	Do not administer KADCYLA. Repeat LVEF assessment within 3 weeks. If LVEF < 40% is confirmed, discontinue KADCYLA.	Do not administer KADCYLA. Repeat LVEF assessment within 3 weeks. If the LVEF has not recovered to within 10% points from baseline, discontinue KADCYLA.	Continue treatment with KADCYLA. Repeat LVEF assessment within 3 weeks.	Continue treatment with KADCYLA.

CHF = Congestive Heart Failure; LVEF = Left Ventricular Ejection Fraction

Thrombocytopenia [see Warnings and Precautions (5.6)]

A reduction in dose is recommended in the case of Grade 4 thrombocytopenia (platelets < 25,000/mm^3) (see Table 5).

Table 5 Dose Modification Guidelines for Thrombocytopenia

Grade 3	Grade 4
PLT 25,000/mm^3 to < 50,000/mm^3	**PLT < 25,000/mm^3**
Do not administer KADCYLA until platelet count recovers to ≤ Grade 1 (≥ 75,000/mm^3), and then treat at same dose level.	Do not administer KADCYLA until platelet count recovers to ≤ Grade 1 (≥ 75,000/mm^3), and then reduce one dose level.

PLT = Platelets

Pulmonary Toxicity [see Warnings and Precautions (5.4)]

KADCYLA should be permanently discontinued in patients diagnosed with interstitial lung disease (ILD) or pneumonitis.

Peripheral Neuropathy [see Warnings and Precautions (5.7)]

KADCYLA should be temporarily discontinued in patients experiencing Grade 3 or 4 peripheral neuropathy until resolution to ≤ Grade 2.

2.3 Preparation for Administration

In order to prevent medication errors it is important to check the vial labels to ensure that the drug being prepared and administered is KADCYLA (ado-trastuzumab emtansine) and not trastuzumab.

Administration:

- Administer KADCYLA as an intravenous infusion only with a 0.22 micron in-line non-protein adsorptive polyethersulfone (PES) filter. Do not administer as an intravenous push or bolus.
- Do not mix KADCYLA, or administer as an infusion, with other medicinal products.
- In order to improve traceability of biological medicinal products, the tradename of the administered product should be clearly recorded (or stated) in the patient file.

Reconstitution:

- Use aseptic technique for reconstitution and preparation of dosing solution. Appropriate procedures for the preparation of chemotherapeutic drugs should be used.
- Using a sterile syringe, slowly inject 5 mL of Sterile Water for Injection into the 100 mg KADCYLA vial, or 8 mL of Sterile Water for Injection into the 160 mg KADCYLA vial to yield a solution containing 20 mg/mL. Swirl the vial gently until completely dissolved. *Do not shake.* Inspect the reconstituted solution for particulates and discoloration.
- The reconstituted solution should be clear to slightly opalescent and free of visible particulates. The color of the reconstituted solution should be colorless to pale brown. Do not use if the reconstituted solution contains visible particulates or is cloudy or discolored.
- The reconstituted lyophilized vials should be used immediately following reconstitution with Sterile Water for Injection. If not used immediately, the reconstituted KADCYLA vials can be stored for up to 4 hours in a refrigerator at 2°C to 8°C (36°F to 46°F); discard unused KADCYLA after 4 hours. *Do not freeze.*
- The reconstituted product contains no preservative and is intended for single-use only.

Dilution:

Determine the correct dose (mg) of KADCYLA *[see Dosage and Administration (2.1)]*.

- Calculate the volume of the 20 mg/mL reconstituted KADCYLA solution needed.
- Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% Sodium Chloride Injection. *Do not use Dextrose (5%) solution.*
- Gently invert the bag to mix the solution in order to avoid foaming.
- The diluted KADCYLA infusion solution should be used immediately. If not used immediately, the solution may be stored in a refrigerator at 2°C to 8°C (36°F to 46°F) for up to 4 hours prior to use. *Do not freeze or shake.*

3 DOSAGE FORMS AND STRENGTHS

Lyophilized powder in single-use vials: 100 mg per vial or 160 mg per vial of ado-trastuzumab emtansine.

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

5.1 Hepatotoxicity

Hepatotoxicity, predominantly in the form of asymptomatic, transient increases in the concentrations of serum transaminases, has been observed in clinical trials with KADCYLA *[see Adverse Reactions (6.1)]*. Serious hepatobiliary disorders, including at least two fatal cases of severe drug-induced liver injury and associated hepatic encephalopathy, have been reported in

clinical trials with KADCYLA. Some of the observed cases may have been confounded by comorbidities and/or concomitant medications with known hepatotoxic potential.

Monitor serum transaminases and bilirubin prior to initiation of KADCYLA treatment and prior to each KADCYLA dose. Patients with known active hepatitis B virus or hepatitis C virus were excluded from Study 1 *[see Clinical Studies (14.1)]*. Reduce the dose or discontinue KADCYLA as appropriate in cases of increased serum transaminases and/or total bilirubin *[see Dosage and Administration (2.2)]*. Permanently discontinue KADCYLA treatment in patients with serum transaminases > 3 x ULN and concomitant total bilirubin > 2 x ULN. KADCYLA has not been studied in patients with serum transaminases > 2.5 x ULN or bilirubin > 1.5 x ULN prior to the initiation of treatment.

In clinical trials of KADCYLA, cases of nodular regenerative hyperplasia (NRH) of the liver have been identified from liver biopsies (3 cases out of 884 treated patients). Two of these three cases of NRH were observed in the randomized trial (Study 1) *[see Adverse Reactions (6.1)]*. NRH is a rare liver condition characterized by widespread benign transformation of hepatic parenchyma into small regenerative nodules; NRH may lead to non-cirrhotic portal hypertension. The diagnosis of NRH can be confirmed only by histopathology. NRH should be considered in all patients with clinical symptoms of portal hypertension but with normal transaminases and no manifestations of cirrhosis. Upon diagnosis of NRH, KADCYLA treatment must be permanently discontinued.

5.2 Left Ventricular Dysfunction

Patients treated with KADCYLA are at increased risk of developing left ventricular dysfunction. A decrease of LVEF to < 40% has been observed in patients treated with KADCYLA. In the randomized trial (Study 1), left ventricular dysfunction occurred in 1.8% of patients in the KADCYLA-treated group and 3.3% of patients in the lapatinib plus capecitabine-treated group *[see Adverse Reactions (6.1)]*.

Assess LVEF prior to initiation of KADCYLA and at regular intervals (e.g. every three months) during treatment to ensure the LVEF is within the institution's normal limits. Treatment with KADCYLA has not been studied in patients with LVEF < 50% prior to initiation of treatment. If, at routine monitoring, LVEF is < 40%, or is 40% to 45% with a 10% or greater absolute decrease below the pretreatment value, withhold KADCYLA and repeat LVEF assessment within approximately 3 weeks. Permanently discontinue KADCYLA if the LVEF has not improved or has declined further *[see Dosage and Administration (2.2)]*. Patients with a history of symptomatic congestive heart failure (CHF), serious cardiac arrhythmia, or history of myocardial infarction or unstable angina within 6 months were excluded from Study 1 *[see Clinical Studies (14.1)]*.

5.3 Embryo-Fetal Toxicity

KADCYLA can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of KADCYLA in pregnant women and no reproductive and developmental toxicology studies have been conducted with ado-trastuzumab emtansine. Nevertheless, treatment with trastuzumab, the antibody component of KADCYLA, during pregnancy in the postmarketing setting has resulted in oligohydramnios, some associated with fatal pulmonary hypoplasia, skeletal abnormalities and neonatal death. DM1, the cytotoxic component of KADCYLA, can be expected to cause embryo-fetal toxicity based on its mechanism of action.

If KADCYLA is used during pregnancy, or if the patient becomes pregnant while receiving KADCYLA, apprise the patient of the potential hazard to the fetus *[see Use in Specific Populations (8.1)]*.

Verify pregnancy status prior to the initiation of KADCYLA. Advise patients of the risks of embryo-fetal death and birth defects and the need for contraception during and after treatment. Advise patients to contact their healthcare provider immediately if they suspect they may be pregnant. If KADCYLA is administered during pregnancy or if a patient becomes pregnant while receiving KADCYLA, immediately report exposure to the Genentech Adverse Event Line at 1-888-835-2555. Encourage women who may be exposed during pregnancy to enroll in the MotHER Pregnancy Registry by contacting 1-800-690-6720 *[see Patient Counseling Information (17)]*.

5.4 Pulmonary Toxicity

Cases of interstitial lung disease (ILD), including pneumonitis, some leading to acute respiratory distress syndrome or fatal outcome have been reported in clinical trials with KADCYLA. Pneumonitis at an incidence of 0.8% (7 out of 884 treated patients) has been reported, with one case of grade 3 pneumonitis. Signs and symptoms include dyspnea, cough, fatigue, and pulmonary infiltrates. These events may or may not occur as sequelae of infusion reactions. In the randomized trial (Study 1), the overall frequency of pneumonitis was 1.2% *[see Adverse Reactions (6.1)]*.

Permanently discontinue treatment with KADCYLA in patients diagnosed with ILD or pneumonitis.

Patients with dyspnea at rest due to complications of advanced malignancy and co-morbidities may be at increased risk of pulmonary toxicity.

5.5 Infusion-Related Reactions, Hypersensitivity Reactions

Treatment with KADCYLA has not been studied in patients who had trastuzumab permanently discontinued due to infusion-related reactions (IRR) and/or hypersensitivity; treatment with KADCYLA is not recommended for these patients.

Infusion-related reactions, characterized by one or more of the following symptoms – flushing, chills, pyrexia, dyspnea, hypotension, wheezing, bronchospasm, and tachycardia have been reported in clinical trials of KADCYLA. In the randomized trial (Study 1), the overall frequency of IRRs in patients treated with KADCYLA was 1.4% *[see Adverse Reactions (6.1)]*. In most patients, these reactions resolved over the course of several hours to a day after the infusion was terminated. KADCYLA treatment should be interrupted in patients with severe IRR. KADCYLA treatment should be permanently discontinued in the event of a life-threatening IRR *[see Dosage and Administration (2.2)]*. Patients should be observed closely for IRR reactions, especially during the first infusion.

One case of a serious, allergic/anaphylactic-like reaction has been observed in clinical trials of single-agent KADCYLA. Medications to treat such reactions, as well as emergency equipment, should be available for immediate use.

5.6 Thrombocytopenia

Thrombocytopenia, or decreased platelet count, was reported in clinical trials of KADCYLA (103 of 884 treated patients with ≥ Grade 3; 283 of 884 treated patients with any Grade). The majority of these patients had Grade 1 or 2 events (< LLN to ≥ 50,000/mm^3) with the nadir occurring by day 8 and generally improving to Grade 0 or 1 (≥ 75,000 /mm^3) by the next scheduled dose. In clinical trials of KADCYLA, the incidence and severity of thrombocytopenia were higher in Asian patients. Independent of race, the incidence of severe hemorrhagic events in patients treated with KADCYLA was low.

In the randomized trial (Study 1), the overall frequency of thrombocytopenia was 31.2% in the KADCYLA-treated group and 3.3% in the lapatinib plus capecitabine-treated group *[see Adverse*

Reactions (6.1)]. The incidence of ≥ Grade 3 thrombocytopenia was 14.5% in the KADCYLA-treated group and 0.4% in the lapatinib plus capecitabine-treated group. In Asian patients, the incidence of ≥ Grade 3 thrombocytopenia was 45.1% in the KADCYLA-treated group and 1.3% in the lapatinib plus capecitabine-treated group.

Monitor platelet counts prior to initiation of KADCYLA and prior to each KADCYLA dose *[see Dosage and Administration (2.2)]*. KADCYLA has not been studied in patients with platelet counts <100,000/mm^3 prior to initiation of treatment. In the event of decreased platelet count to Grade 3 or greater (< 50,000/mm^3) do not administer KADCYLA until platelet counts recover to Grade 1 (≥ 75,000/mm^3) *[see Dosage and Administration (2.2)]*. Patients with thrombocytopenia (< 100,000/mm^3) and patients on anti-coagulant treatment should be closely monitored during treatment with KADCYLA.

5.7 Neurotoxicity

Peripheral neuropathy, mainly as Grade 1 and predominantly sensory, was reported in clinical trials of KADCYLA (14 of 884 treated patients with ≥ Grade 3; 196 of 884 treated patients with any Grade). In the randomized trial (Study 1), the overall frequency of peripheral neuropathy was 21.2% in the KADCYLA-treated group and 13.5% in the lapatinib plus capecitabine-treated group *[see Adverse Reactions (6.1)]*. The incidence of ≥ Grade 3 peripheral neuropathy was 2.2% in the KADCYLA-treated group and 0.2% in the lapatinib plus capecitabine-treated group.

KADCYLA should be temporarily discontinued in patients experiencing Grade 3 or 4 peripheral neuropathy until resolution to ≤ Grade 2. Patients should be clinically monitored on an ongoing basis for signs or symptoms of neurotoxicity *[see Nonclinical Toxicology (13.2)]*.

5.8 HER2 Testing

Detection of HER2 protein overexpression or gene amplification is necessary for selection of patients appropriate for KADCYLA therapy because these are the only patients studied for whom benefit has been shown *[see Indications and Usage (1), Clinical Studies (14.1)]*. In the randomized study (Study 1), patients with breast cancer were required to have evidence of HER2 overexpression defined as 3+ IHC by Dako Herceptest™ or evidence of overexpression defined as FISH amplification ratio ≥ 2.0 by Dako *HER2* FISH PharmDx™ test kit. Only limited data were available for patients whose breast cancer was positive by FISH and 0 or 1+ by IHC.

Assessment of HER2 status should be performed by laboratories with demonstrated proficiency in the specific technology being utilized. Improper assay performance, including use of sub-optimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.

5.9 Extravasation

In KADCYLA clinical studies, reactions secondary to extravasation have been observed. These reactions, observed more frequently within 24 hours of infusion, were usually mild and comprised erythema, tenderness, skin irritation, pain, or swelling at the infusion site. Specific treatment for KADCYLA extravasation is unknown. The infusion site should be closely monitored for possible subcutaneous infiltration during drug administration.

6 ADVERSE REACTIONS

The following adverse reactions are discussed in greater detail in other sections of the label:

- Hepatotoxicity *[See Warnings and Precautions (5.1)]*
- Left Ventricular Dysfunction *[See Warnings and Precautions (5.2)]*
- Embryo-Fetal Toxicity *[See Warnings and Precautions (5.3)]*

- Pulmonary Toxicity *[See Warnings and Precautions (5.4)]*
- Infusion-Related Reactions, Hypersensitivity Reactions *[See Warnings and Precautions (5.5)]*
- Thrombocytopenia *[See Warnings and Precautions (5.6)]*
- Neurotoxicity *[See Warnings and Precautions (5.7)]*

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

In clinical trials, KADCYLA has been evaluated as single-agent in 884 patients with HER2-positive metastatic breast cancer. The most common (frequency ≥ 25%) adverse drug reactions (ADRs) seen in 884 patients treated with KADCYLA were fatigue, nausea, musculoskeletal pain, thrombocytopenia, headache, increased transaminases, and constipation.

The ADRs described in Table 6 were identified in patients with HER2-positive metastatic breast cancer treated in a randomized trial (Study 1) *[see Clinical Studies (14.1)]*. Patients were randomized to receive KADCYLA or lapatinib plus capecitabine. The median duration of study treatment was 7.6 months for patients in the KADCYLA-treated group and 5.5 months and 5.3 months for patients treated with lapatinib and capecitabine, respectively. Two hundred and eleven (43.1%) patients experienced ≥ Grade 3 adverse events in the KADCYLA-treated group compared with 289 (59.2%) patients in the lapatinib plus capecitabine-treated group. Dose adjustments for KADCYLA were permitted *[see Dosage and Administration (2.2)]*. Thirty-two patients (6.5%) discontinued KADCYLA due to an adverse event, compared with 41 patients (8.4%) who discontinued lapatinib, and 51 patients (10.5%) who discontinued capecitabine due to an adverse event. The most common adverse events leading to KADCYLA withdrawal were thrombocytopenia and increased transaminases. Eighty patients (16.3%) treated with KADCYLA had adverse events leading to dose reductions. The most frequent adverse events leading to dose reduction of KADCYLA (in ≥ 1% of patients) included thrombocytopenia, increased transaminases, and peripheral neuropathy. Adverse events that led to dose delays occurred in 116 (23.7%) of KADCYLA treated patients. The most frequent adverse events leading to a dose delay of KADCYLA (in ≥ 1% of patients) were neutropenia, thrombocytopenia, leukopenia, fatigue, increased transaminases and pyrexia.

Table 6 reports the ADRs that occurred in patients in the KADCYLA-treated group (n=490) of the randomized trial (Study 1). Selected laboratory abnormalities are shown in Table 7. The most common ADRs seen with KADCYLA in the randomized trial (frequency > 25%) were nausea, fatigue, musculoskeletal pain, thrombocytopenia, increased transaminases, headache, and constipation. The most common NCI–CTCAE (version 3) ≥ Grade 3 ADRs (frequency >2%) were thrombocytopenia, increased transaminases, anemia, hypokalemia, peripheral neuropathy and fatigue.

Table 6 Summary of Adverse Drug Reactions Occurring in Patients on the KADCYLA Treatment Arm in the Randomized Trial (Study 1)

Adverse Drug Reactions (MedDRA) System Organ Class	KADCYLA (3.6 mg/kg) n=490 Frequency rate %		Lapatinib (1250 mg) + Capecitabine (2000 mg/m^2) n=488 Frequency rate %	
	All grades (%)	Grade 3 – 4 (%)	All grades (%)	Grade 3 – 4 (%)
Blood and Lymphatic System Disorders				
Neutropenia	6.7	2.0	9.0	4.3
Anemia	14.3	4.1	10.5	2.5
Thrombocytopenia	31.2	14.5	3.3	0.4
Cardiac Disorders				
Left ventricular dysfunction	1.8	0.2	3.3	0.4
Eye Disorders				
Lacrimation increased	3.3	0	2.5	0
Dry eye	3.9	0	3.1	0
Vision blurred	4.5	0	0.8	0
Conjunctivitis	3.9	0	2.3	0
Gastrointestinal Disorders				
Dyspepsia	9.2	0	11.5	0.4
Stomatitis	14.1	0.2	32.6	2.5
Dry Mouth	16.7	0	4.9	0.2
Abdominal pain	18.6	0.8	17.6	1.6
Vomiting	19.2	0.8	29.9	4.5
Diarrhea	24.1	1.6	79.7	20.7
Constipation	26.5	0.4	11.1	0
Nausea	39.8	0.8	45.1	2.5
General Disorders and Administration				
Peripheral edema	7.1	0	8.2	0.2
Chills	7.6	0	3.1	0
Pyrexia	18.6	0.2	8.4	0.4
Asthenia	17.8	0.4	17.6	1.6
Fatigue	36.3	2.5	28.3	3.5
Hepatobiliary Disorders				
Nodular regenerative hyperplasia*	0.4	ND	0	0
Portal hypertension*	0.4	0.2	0	0
Immune System Disorders				
Drug hypersensitivity	2.2	0	0.8	0
Injury, Poisoning, and Procedural				
Infusion-related reaction	1.4	0	0.2	0
Infections and Infestations				
Urinary tract infection	9.4	0.6	3.9	0
Investigations				
Blood alkaline phosphatase	4.7	0.4	3.7	0.4

Adverse Drug Reactions (MedDRA) System Organ Class	KADCYLA (3.6 mg/kg) n=490 Frequency rate %		Lapatinib (1250 mg) + Capecitabine (2000 mg/m^2) n=488 Frequency rate %	
	All grades (%)	Grade 3 – 4 (%)	All grades (%)	Grade 3 – 4 (%)
increased				
Increased transaminases	28.8	8.0	14.3	2.5
Metabolism and Nutrition Disorders				
Hypokalemia	10.2	2.7	9.4	4.7
Musculoskeletal and Connective Tissue Disorders				
Myalgia	14.1	0.6	3.7	0
Arthralgia	19.2	0.6	8.4	0
Musculoskeletal pain	36.1	1.8	30.5	1.4
Nervous System Disorders				
Dysgeusia	8.0	0	4.1	0.2
Dizziness	10.2	0.4	10.7	0.2
Peripheral neuropathy	21.2	2.2	13.5	0.2
Headache	28.2	0.8	14.5	0.8
Psychiatric Disorders				
Insomnia	12.0	0.4	8.6	0.2
Respiratory, Thoracic, and Mediastinal Disorders				
Pneumonitis	1.2	0	0	0
Dyspnea	12.0	0.8	8.0	0.4
Cough	18.2	0.2	13.1	0.2
Epistaxis	22.5	0.2	8.4	0
Skin and Subcutaneous Tissue Disorders				
Pruritus	5.5	0.2	9.2	0
Rash	11.6	0	27.5	1.8
Vascular Disorders				
Hypertension	5.1	1.2	2.3	0.4

* Nodular Regenerative Hyperplasia and Portal Hypertension occurred in the same patient.

ND = Not determined

Table 7 Selected Laboratory Abnormalities

Parameter	KADCYLA (3.6 mg/kg)			Lapatinib (1250 mg) + Capecitabine (2000 mg/m^2)		
	All Grade %	Grade 3 %	Grade 4 %	All Grade %	Grade 3 %	Grade 4 %
Increased bilirubin	17	<1	0	57	2	0
Increased AST	98	7	<1	65	3	0
Increased ALT	82	5	<1	54	3	0
Decreased platelet count	83	14	3	21	<1	<1
Decreased hemoglobin	60	4	1	64	3	<1
Decreased neutrophils	39	3	<1	38	6	2
Decreased potassium	33	3	0	31	6	<1

6.2 Immunogenicity

As with all therapeutic proteins, there is the potential for an immune response to KADCYLA. A total of 836 patients from six clinical studies were tested at multiple time points for anti-therapeutic antibody (ATA) responses to KADCYLA. Following KADCYLA dosing, 5.3% (44/836) of patients tested positive for anti-KADCYLA antibodies at one or more post-dose time points. The presence of KADCYLA in patient serum at the time of ATA sampling may interfere with the ability of this assay to detect anti-KADCYLA antibodies. As a result, data may not accurately reflect the true incidence of anti-KADCYLA antibody development. In addition, neutralizing activity of anti-KADCYLA antibodies has not been assessed.

Immunogenicity data are highly dependent on the sensitivity and specificity of the test methods used. Additionally, the observed incidence of a positive result in a test method may be influenced by several factors, including sample handling, timing of sample collection, drug interference, concomitant medication and the underlying disease. Therefore, comparison of the incidence of antibodies to KADCYLA with the incidence of antibodies to other products may be misleading. Clinical significance of anti-KADCYLA antibodies is not yet known.

7 DRUG INTERACTIONS

No formal drug-drug interaction studies with KADCYLA have been conducted. *In vitro* studies indicate that DM1, the cytotoxic component of KADCYLA, is metabolized mainly by CYP3A4 and to a lesser extent by CYP3A5. Concomitant use of strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole) with KADCYLA should be avoided due to the potential for an increase in DM1 exposure and toxicity. Consider an alternate medication with no or minimal potential to inhibit CYP3A4. If concomitant use of strong CYP3A4 inhibitors is unavoidable, consider delaying KADCYLA treatment until the strong CYP3A4 inhibitors have cleared from the circulation (approximately 3 elimination half-lives of the inhibitors) when possible. If a strong CYP3A4 inhibitor is coadministered and KADCYLA treatment cannot be delayed, patients should be closely monitored for adverse reactions.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D *[see Warnings and Precautions (5.3)]*

Risk Summary

KADCYLA can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of KADCYLA in pregnant women. No reproductive and

developmental toxicology studies have been conducted with ado-trastuzumab emtansine. Nevertheless, two components of KADCYLA (trastuzumab and DM1) are known or suspected to cause fetal harm or death when administered to a pregnant woman. If KADCYLA is administered during pregnancy, or if a patient becomes pregnant while receiving KADCYLA, apprise the patient of the potential hazard to the fetus. Patients should be advised to use effective contraception during treatment with KADCYLA and for 6 months following the last dose of KADCYLA.

If KADCYLA is administered during pregnancy or if a patient becomes pregnant while receiving KADCYLA, immediately report exposure to the Genentech Adverse Event Line at 1-888-835-2555. Encourage women who may be exposed during pregnancy to enroll in the MotHER Pregnancy Registry by contacting 1-800-690-6720 *[see Patient Counseling Information (17)]*.

Human Data

In the post-marketing setting, treatment with trastuzumab during pregnancy has resulted in cases of oligohydramnios, some associated with fatal pulmonary hypoplasia, skeletal abnormalities and neonatal death. These case reports described oligohydramnios in pregnant women who received trastuzumab either alone or in combination with chemotherapy. In some case reports, amniotic fluid index increased after trastuzumab was stopped. In one case, trastuzumab therapy resumed after the amniotic fluid index improved, and oligohydramnios recurred.

Animal Data

There were no reproductive and developmental toxicology studies conducted with ado-trastuzumab emtansine. DM1, the cytotoxic component of KADCYLA, disrupts microtubule function. DM1 is toxic to rapidly dividing cells in animals and is genotoxic, suggesting it has the potential to cause embryotoxicity and teratogenicity. In studies where trastuzumab was administered to pregnant monkeys at doses up to 25 mg/kg (about 7 times the clinical dose), trastuzumab crossed the placental barrier during the early and late phases of gestation. The resulting concentrations of trastuzumab in fetal blood and amniotic fluid were approximately 33% and 25%, respectively, of those present in the maternal serum but were not associated with adverse findings.

8.3 Nursing Mothers

It is not known whether KADCYLA, specifically, is excreted in human milk, but IgG is known to be excreted in human milk. In lactating monkeys, trastuzumab was excreted in small amounts (about 0.3% of maternal serum concentrations) in breast milk after post-partum doses of 25 mg/kg (about 7 times the clinical dose of KADCYLA). Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from KADCYLA, a decision should be made whether to discontinue nursing or discontinue KADCYLA, taking into account the importance of the drug to the mother *[see Warnings and Precautions (5.3)]*.

8.4 Pediatric Use

Safety and effectiveness of KADCYLA have not been established in pediatric patients.

8.5 Geriatric Use

Of 495 patients who were randomized to KADCYLA in the randomized trial (Study 1) *[see Clinical Studies (14.1)]*, 65 patients (13%) were ≥ 65 years of age and 11 patients (2%) were ≥ 75 years of age. In patients ≥ 65 years old (n=138 across both treatment arms) the hazard ratios for progression-free survival (PFS) and Overall Survival (OS) were 1.06 (95% CI: 0.68, 1.66) and 1.05 (95% CI: 0.58, 1.91), respectively.

Population pharmacokinetic analysis indicates that age does not have a clinically meaningful effect on the pharmacokinetics of ado-trastuzumab emtansine *[see Clinical Pharmacology (12.3)]*.

8.6 Females of Reproductive Potential

KADCYLA can cause embryo-fetal harm when administered during pregnancy. Counsel patients regarding pregnancy prevention and planning. Advise females of reproductive potential to use effective contraception while receiving KADCYLA and for 6 months following the last dose of KADCYLA.

If KADCYLA is administered during pregnancy or if the patient becomes pregnant while receiving KADCYLA, immediately report exposure to the Genentech Adverse Event Line at 1-888-835-2555. Encourage women who may be exposed during pregnancy to enroll in the MotHER Pregnancy Registry by contacting 1-800-690-6720 *[see Patient Counseling Information (17)]*.

8.7 Renal Impairment

No dedicated renal impairment trial for KADCYLA has been conducted. Based on the population pharmacokinetics, as well as analysis of Grade 3 or greater adverse drug reactions and dose modifications, dose adjustments of KADCYLA are not needed in patients with mild (creatinine clearance [CLcr] 60 to 89 mL/min) or moderate (CLcr 30 to 59 mL/min) renal impairment. No dose adjustment can be recommended for patients with severe renal impairment (CLcr less than 30 mL/min) because of the limited data available *[see Clinical Pharmacology (12.3)]*.

8.8 Hepatic Impairment

In vitro studies in human liver microsomes indicates that DM1 is metabolized by CYP3A4/5. The influence of hepatic impairment on the pharmacokinetics of ado-trastuzumab emtansine conjugate has not been determined.

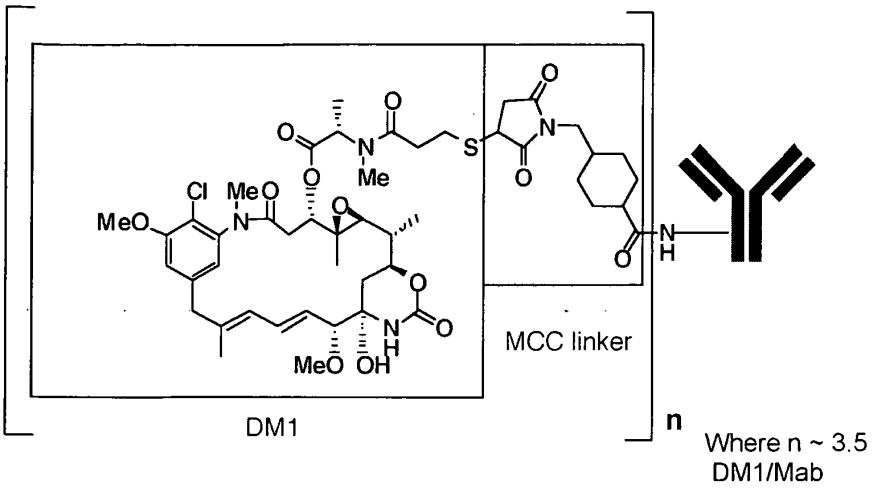
10 OVERDOSAGE

There is no known antidote for overdose of KADCYLA. In clinical trials, overdose of KADCYLA has been reported at approximately two times the recommended dose which resulted in Grade 2 thrombocytopenia (resolved 4 days later) and one death. In the fatal case, the patient incorrectly received KADCYLA at 6 mg/kg and died approximately 3 weeks following the overdose; a cause of death and a causal relationship to KADCYLA were not established.

11 DESCRIPTION

KADCYLA (ado-trastuzumab emtansine) is a HER2-targeted antibody-drug conjugate (ADC) which contains the humanized anti-HER2 IgG1, trastuzumab, covalently linked to the microtubule inhibitory drug DM1 (a maytansine derivative) via the stable thioether linker MCC (4-[N-maleimidomethyl] cyclohexane-1-carboxylate). Emtansine refers to the MCC-DM1 complex.

The antibody trastuzumab, is a well characterized recombinant monoclonal antibody product produced by mammalian (Chinese hamster ovary) cells, and the small molecule components (DM1 and MCC) are produced by chemical synthesis. Ado-trastuzumab emtansine contains an average of 3.5 DM1 molecules per antibody. Ado-trastuzumab emtansine has the following chemical structure:



Note: The bracketed structure is DM1 plus MCC which represents the emtansine component. The n is, on average, 3.5 DM1 molecules per trastuzumab (Mab) molecule.

KADCYLA (ado-trastuzumab emtansine) is a sterile, white to off-white preservative free lyophilized powder in single-use vials. Each vial contains 100 mg or 160 mg ado-trastuzumab emtansine. Following reconstitution, each single-use vial contains ado-trastuzumab emtansine (20 mg/mL), polysorbate 20 [0.02% (w/v)], sodium succinate (10 mM), and sucrose [6% (w/v)] with a pH of 5.0 and density of 1.026 g/mL. The resulting solution containing 20 mg/mL ado-trastuzumab emtansine is administered by intravenous infusion following dilution.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Ado-trastuzumab emtansine is a HER2-targeted antibody-drug conjugate. The antibody is the humanized anti-HER2 IgG1, trastuzumab. The small molecule cytotoxin, DM1, is a microtubule inhibitor. Upon binding to sub-domain IV of the HER2 receptor, ado-trastuzumab emtansine undergoes receptor-mediated internalization and subsequent lysosomal degradation, resulting in intracellular release of DM1-containing cytotoxic catabolites. Binding of DM1 to tubulin disrupts microtubule networks in the cell, which results in cell cycle arrest and apoptotic cell death. In addition, *in vitro* studies have shown that similar to trastuzumab, ado-trastuzumab emtansine inhibits HER2 receptor signaling, mediates antibody-dependent cell-mediated cytotoxicity and inhibits shedding of the HER2 extracellular domain in human breast cancer cells that overexpress HER2.

12.3 Pharmacokinetics

The pharmacokinetics of KADCYLA was evaluated in a phase 1 study and in a population pharmacokinetic analysis for the ado-trastuzumab emtansine conjugate (ADC) using pooled data from 5 trials in patients with breast cancer. A linear two-compartment model with first-order elimination from the central compartment adequately describes the ADC concentration-time profile. In addition to ADC, the pharmacokinetics of total antibody (conjugated and unconjugated trastuzumab), DM1 were also determined. The pharmacokinetics of KADCYLA are summarized below.

Distribution

Maximum concentrations (C_{max}) of ADC and DM1 were observed close to the end of infusion. In Study 1, mean (SD) ADC and DM1 Cycle 1 C_{max} following KADCYLA administration was 83.4 (16.5) µg/mL and 4.61 (1.61) ng/mL, respectively.

In vitro, the mean binding of DM1 to human plasma proteins was 93%. *In vitro,* DM1 was a substrate of P-glycoprotein (P-gp).

Based on population pharmacokinetic analysis, the central volume of distribution of ADC was 3.13 L.

Metabolism

In vitro studies indicate that DM1, the small molecule component of KADCYLA, undergoes metabolism by CYP3A4/5. DM1 did not inhibit or induce major CYP450 enzymes *in vitro.* In human plasma, ado-trastuzumab emtansine catabolites MCC-DM1, Lys-MCC-DM1, and DM1 were detected at low levels.

Elimination

Based on population pharmacokinetic analysis, following intravenous infusion of KADCYLA, the clearance of the ADC was 0.68 L/day and the elimination half-life ($t_{1/2}$) was approximately 4 days. No accumulation of KADCYLA was observed after repeated dosing of intravenous infusion every 3 weeks.

Based on population pharmacokinetic analysis (n=671), body weight, sum of longest diameter of target lesions by RECIST, HER2 extracellular domain (ECD) concentrations, AST, albumin, and baseline trastuzumab concentrations were identified as statistically significant covariates for ado-trastuzumab emtansine clearance. However, the magnitude of effect of these covariates on ado-trastuzumab emtansine exposure suggests that, with the exception of body weight, these covariates are unlikely to have a clinically meaningful effect on KADCYLA exposure. Therefore, the body weight based dose of 3.6 mg/kg every 3 weeks without correction for other covariates is considered appropriate.

Effect of Renal Impairment

Based on population pharmacokinetic analysis in 668 patients, including moderate (CL_{cr} 30 - 59 mL/min, n=53) and mild (CL_{cr} 60 - 89 mL/min, n=254) renal impairment, indicate that pharmacokinetics of the ADC is not affected by mild to moderate renal impairment as compared to normal renal function (CLcr ≥ 90 mL/min, n=361). Data from only one patient with severe renal impairment (CL_{cr} < 30 mL/min) is available *[see Use in Specific Populations (8.7)]*.

Effects of Age and Race

Based on population pharmacokinetic analysis, age (< 65 (n=577); 65 - 75 (n=78); > 75 (n=16)) and race (Asian (n=73); non-Asian (n=598)) do not have a clinically meaningful effect on the pharmacokinetics of ado-trastuzumab emtansine.

12.6 Cardiac Electrophysiology

The effect of multiple doses of KADCYLA (3.6 mg/kg every 3 weeks) on the QTc interval was evaluated in an open label, single arm study in 51 patients with HER2-positive metastatic breast cancer. No large changes in the mean QT interval (i.e., > 20 ms) were detected in the study.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies have not been conducted with ado-trastuzumab emtansine.

DM1 was aneugenic or clastogenic in an *in vivo* single-dose rat bone marrow micronucleus assay at exposures that were comparable to mean maximum concentrations of DM1 measured in humans administered KADCYLA. DM1 was not mutagenic in an *in vitro* bacterial reverse mutation (Ames) assay.

Based on results from animal toxicity studies, KADCYLA may impair fertility in humans. In a single-dose toxicity study of ado-trastuzumab emtansine in rats, degeneration of seminiferous tubules with hemorrhage in the testes associated with increased weights of testes and epididymides at a severely toxic dose level (60 mg/kg; about 4 times the clinical exposure based on AUC) were observed. The same dose in female rats resulted in signs of hemorrhage and necrosis of the corpus luteum in ovaries. In monkeys dosed with ado-trastuzumab emtansine once every three weeks for 12 weeks (four doses), at up to 30 mg/kg (about 7 times the clinical exposure based on AUC), there were decreases in the weights of epididymides, prostate, testes, seminal vesicles and uterus, although the interpretation of these effects is unclear due to the varied sexual maturity of enrolled animals.

13.2 Animal Toxicology and/or Pharmacology

In monkeys, treatment with doses of ado-trastuzumab emtansine up to 30 mg/kg (about 7 times the clinical exposure based on AUC) caused dose dependent axonal degeneration in the sciatic nerve with hypertrophy or hyperplasia of the Schwann cells, and axonal degeneration of the dorsal funiculus in the spinal cord. Based on the mechanism of action of the cytotoxic component DM1, there is clinical potential for neurotoxicity *[see Warnings and Precautions (5.7)]*.

14 CLINICAL STUDIES

14.1 Metastatic Breast Cancer

The efficacy of KADCYLA was evaluated in a randomized, multicenter, open-label trial of 991 patients with HER2-positive, unresectable locally advanced or metastatic breast cancer. Prior taxane and trastuzumab-based therapy was required before trial enrollment. Patients with only prior adjuvant therapy were required to have disease recurrence during or within six months of completing adjuvant therapy. Breast tumor samples were required to show HER2 overexpression defined as 3+ IHC or FISH amplification ratio $\geq$ 2.0 determined at a central laboratory. Patients were randomly allocated (1:1) to receive lapatinib plus capecitabine or KADCYLA. Randomization was stratified by world region (United States, Western Europe, other), number of prior chemotherapy regimens for unresectable locally advanced or metastatic disease (0–1, >1) and visceral versus non-visceral disease as determined by the investigators.

KADCYLA was given intravenously at 3.6 mg/kg on Day 1 of a 21-day cycle. Lapatinib was administered at 1250 mg/day orally once per day of a 21-day cycle and capecitabine was administered at 1000 mg/m^2 orally twice daily on Days 1–14 of a 21-day cycle. Patients were treated with KADCYLA or lapatinib plus capecitabine until progression of disease, withdrawal of consent, or unacceptable toxicity. At the time of the primary analysis, median time on study drug was 5.7 months (range: 0–28.4) for KADCYLA, 4.9 months (range: 0–30.8) for lapatinib, and 4.8 months (range: 0–30.4) for capecitabine.

The co-primary efficacy endpoints of the study were progression-free survival (PFS) based on tumor response assessments by an independent review committee (IRC), and overall survival (OS). PFS was defined as the time from the date of randomization to the date of disease progression or death from any cause (whichever occurred earlier). Overall survival was defined as the time from the date of randomization to the date of death from any cause. Additional endpoints included PFS (based on investigator tumor response assessments), objective response rate (ORR), duration of response and time to symptom progression.

Patient demographics and baseline tumor characteristics were balanced between treatment arms. All patients had metastatic disease at study entry. The median age was approximately 53 years (range 24-84 years), 74% were White, 18% were Asian and 5% were Black. All but 5 patients were women. Twenty-seven percent of patients were enrolled in United States, 32% in Europe and 16% in Asia. Tumor prognostic characteristics including hormone receptor status (positive:

55%, negative: 43%), presence of visceral disease (68%) and non-visceral disease only (33%) and the number of metastatic sites (< 3: 61%, ≥ 3: 37%) were similar in the study arms.

The majority of patients (88%) had received prior systemic treatment in the metastatic setting. Twelve percent of patients had prior treatment only in the neoadjuvant or adjuvant setting and had disease relapse within 6 months of treatment. All but one patient received trastuzumab prior to study entry; approximately 85% of patients received prior trastuzumab in the metastatic setting. Over 99% percent of patients had received a taxane, and 61% of patients had received an anthracycline prior to study entry. Overall, patients received a median of 3 systemic agents in the metastatic setting. Among patients with hormone receptor-positive tumors, 44.4% received prior adjuvant hormonal therapy and 44.8% received hormonal therapy for locally advanced/metastatic disease.

The randomized trial demonstrated a statistically significant improvement in IRC-assessed PFS in the KADCYLA-treated group compared with the lapatinib plus capecitabine-treated group [hazard ratio (HR) = 0.65, 95% CI: 0.55, 0.77, $p < 0.0001$], and an increase in median PFS of 3.2 months (median PFS of 9.6 months in the KADCYLA-treated group vs. 6.4 months in the lapatinib plus capecitabine group). See Table 8 and Figure 1. The results for investigator-assessed PFS were similar to those observed for IRC-assessed PFS.

At the time of PFS analysis, 223 patients had died. More deaths occurred in the lapatinib plus capecitabine arm (26%) compared with the KADCYLA arm (19%), however the results of this interim OS analysis did not meet the pre-specified stopping boundary for statistical significance. At the time of the second interim OS analysis, 331 events had occurred. The co-primary endpoint of OS was met; OS was significantly improved in patients receiving KADCYLA (HR = 0.68, 95% CI: 0.55, 0.85, $p = 0.0006$). This result crossed the pre-specified efficacy stopping boundary (HR = 0.73 or $p = 0.0037$). The median duration of survival was 30.9 months in the KADCYLA arm vs. 25.1 months in the lapatinib plus capecitabine arm. See Table 8 and Figure 2.

A treatment benefit with KADCYLA in terms of PFS and OS was observed in patient subgroups based on stratification factors, key baseline demographic and disease characteristics, and prior treatments. In the subgroup of patients with hormone receptor-negative disease (n=426), the hazard ratios for PFS and OS were 0.56 (95% CI: 0.44, 0.72) and 0.75 (95% CI: 0.54, 1.03), respectively. In the subgroup of patients with hormone receptor-positive disease (n=545), the hazard ratios for PFS and OS were 0.72 (95% CI: 0.58, 0.91) and 0.62 (95% CI: 0.46, 0.85), respectively. In the subgroup of patients with non-measurable disease (n=205), based on IRC assessments, the hazard ratios for PFS and OS were 0.91 (95% CI: 0.59, 1.42) and 0.96 (95% CI: 0.54, 1.68), respectively; in patients with measurable disease the hazard ratios were 0.62 (95% CI: 0.52, 0.75) and 0.65 (95% CI: 0.51, 0.82), respectively. The PFS and OS hazard ratios in patients who were younger than 65 years old (n=853) were 0.62 (95% CI: 0.52, 0.74) and 0.66 (95% CI: 0.52, 0.83), respectively. In patients ≥ 65 years old (n=138), the hazard ratios for PFS and OS were 1.06 (95% CI: 0.68, 1.66) and 1.05 (95% CI: 0.58, 1.91), respectively.

Table 8 **Summary of Efficacy from Study 1**

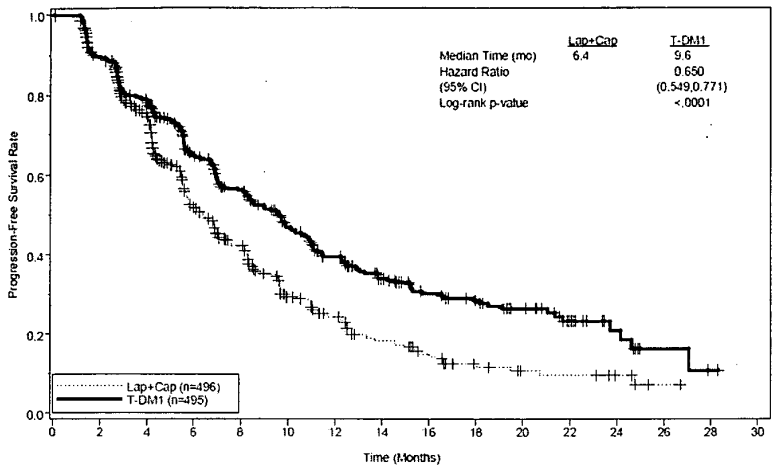
	KADCYLA N= 495	Lapatinib +Capecitabine N= 496
Progression-Free Survival (independent review)		
Number (%) of patients with event	265 (53.5%)	304 (61.3%)
Median duration of PFS (months)	9.6	6.4
Hazard Ratio (stratified*)	0.650	
95% CI for Hazard Ratio	(0.549, 0.771)	
p-value (Log-Rank test, stratified*)	<0.0001	
Overall Survival **		
Number (%) of patients who died	149 (30.1%)	182 (36.7%)
Median duration of survival (months)	30.9	25.1
Hazard Ratio (stratified*)	0.682	
95% CI for Hazard Ratio	(0.548, 0.849)	
p-value (Log-Rank test*)	0.0006	
Objective Response Rate (independent review)		
Patients with measurable disease	397	389
Number of patients with OR (%)	173 (43.6%)	120 (30.8%)
Difference (95% CI)	12.7% (6.0, 19.4)	
Duration of Objective Response (months)		
Number of patients with OR	173	120
Median duration (95% CI)	12.6 (8.4, 20.8)	6.5 (5.5, 7.2)

PFS: progression-free survival; OR: objective response

* Stratified by world region (United States, Western Europe, other), number of prior chemotherapeutic regimens for locally advanced or metastatic disease (0-1 vs. >1), and visceral vs. non-visceral disease.

** The second interim analysis for OS was conducted when 331 events were observed and the results are presented in this table.

Figure 1 Kaplan-Meier Curve of IRC-Assessed Progression-Free Survival for Study 1

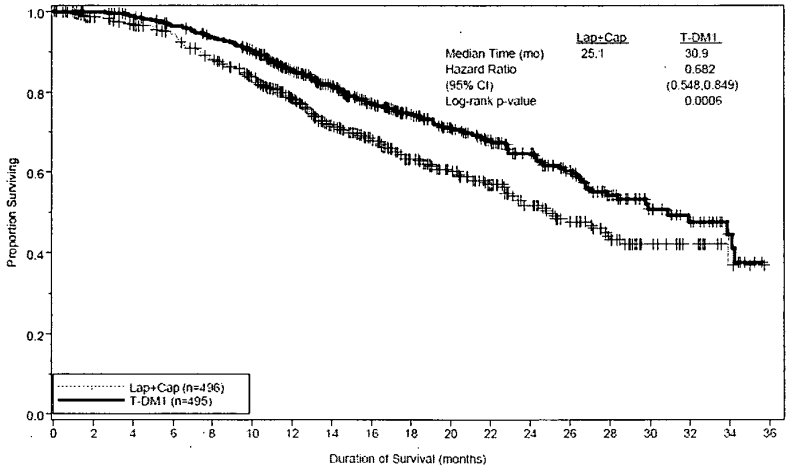


Number at Risk:

Lap+Cap	496	404	310	176	129	73	53	35	25	14	9	8	5	1	0	0
T-DM1	495	419	341	236	183	130	101	72	54	44	30	18	9	3	1	0

T-DM1: ado-trastuzumab emtansine; Lap: lapatinib; Cap: capecitabine; IRC: independent review committee.
Hazard ratio is estimated based on a stratified Cox model; p-value is estimated based on a stratified log-rank test.

Figure 2 Kaplan-Meier Curve of Overall Survival for Study 1



Number at Risk:

Lap+Cap	496	471	453	435	403	368	297	240	204	159	133	110	86	63	45	27	17	7	4
T-DM1	495	485	474	457	439	418	349	293	242	197	164	136	111	86	62	38	28	13	5

T-DM1: ado-trastuzumab emtansine; Lap: lapatinib; Cap: capecitabine.
Hazard ratio is estimated based on a stratified Cox model; p-value is estimated based on a stratified log-rank test.

15 REFERENCES

1. OSHA Hazardous Drugs. *OSHA*. http://www.osha.gov/SLTC/hazardousdrugs/index.html

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied/Storage

KADCYLA (ado-trastuzumab emtansine) is supplied as:

Carton Contents	NDC
One 100 mg vial, single use vial	NDC 50242-088-01
One 160 mg vial, single use vial	NDC 50242-087-01

Store vials in a refrigerator at 2°C to 8°C (36°F to 46°F) until time of reconstitution. *Do not freeze or shake.*

16.2 Special Handling

Follow procedures for proper handling and disposal of anticancer drugs[1].

17 PATIENT COUNSELING INFORMATION

- Inform patients of the possibility of severe liver injury and advise patients to immediately seek medical attention if they experience symptoms of acute hepatitis such as nausea, vomiting, abdominal pain (especially RUQ abdominal pain), jaundice, dark urine, generalized pruritus, anorexia, etc. *[see Warnings and Precautions (5.1)]*.
- Advise patients to contact a health care professional immediately for any of the following: new onset or worsening shortness of breath, cough, swelling of the ankles/legs, palpitations, weight gain of more than 5 pounds in 24 hours, dizziness or loss of consciousness *[see Warnings and Precautions (5.2)]*.
- Advise pregnant women and females of reproductive potential that KADCYLA exposure can result in fetal harm, including embryo-fetal death or birth defects *[see Warnings and Precautions (5.3), Use in Specific Populations (8.1, 8.6)]*.
- Advise females of reproductive potential to use effective contraception while receiving KADCYLA and for 6 months following the last dose of KADCYLA *[See Warnings and Precautions (5.3) and Use in Specific Populations (8.1, 8.6)]*.
- Advise nursing mothers treated with KADCYLA to discontinue nursing or discontinue KADCYLA, taking into account the importance of the drug to the mother *[see Use in Specific Populations (8.3)]*.
- Encourage women who are exposed to KADCYLA during pregnancy to enroll in the MotHER Pregnancy Registry by contacting 1-800-690-6720 *[see Warnings and Precautions (5.3) and Use in Specific Populations (8.1, 8.6)]*.

KADCYLA™ [ado-trastuzumab emtansine]

4862200

Initial U.S. Approval: February 2013

Manufactured by:
Genentech, Inc.
A Member of the Roche Group
1 DNA Way
South San Francisco, CA 94080-4990
U.S. License No: 1048

Attachment C

Ado-trastuzumab emtansine Biologics' License Application (BLA) Approval (redacted)

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125427Orig1s000

APPROVAL LETTER

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

BLA 125427/0

BLA APPROVAL

Genentech, Inc.
Attention: Erica J. Evans, Ph.D.
Regulatory Program Management
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Evans:

Please refer to your Biologics License Application (BLA) dated August 24, 2012, received August 27, 2012, submitted under section 351(a) of the Public Health Service Act for Kadcyla (ado-trastuzumab emtansine).

We acknowledge receipt of your amendments dated June 12, and 25; July 11 and 31; August 24, and 27; September 12, 18(2), 21, 25(2), 26(2), and 28(2); October 8(2), 9(2), 11(2), 17(2), 18(2), 23(2), 24, 25, 29, 30, and 31; November 1, 2(3), 5, 6, 8(2), 12(3), 13(3), 14(2), 16, 20(2), 26, and 30(2); December 5(2), 6, 7(6), 13, 14, 19, 20, 21(2) and January 2, 3, 4, 7, 11, 15(2), 17(2), 18, 22, 23, 24,(3), 25(3), 28(2), 30(2) and February 5, 6, 7, 8, 12, and 15, 2013.

LICENSING

We have approved your BLA for Kadcyla (ado-trastuzumab emtansine) effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, Kadcyla (ado-trastuzumab emtansine) under your existing Department of Health and Human Services U.S. License No. 1048. Kadcyla (ado-trastuzumab emtansine) is indicated, as a single agent, for the treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination.

MANUFACTURING LOCATIONS

Under this license, you are approved to manufacture ado-trastuzumab emtansine bulk drug substance at (b)(4), and ado-trastuzumab emtansine final drug product at (b)(4). Drug product labeling and packaging will be done at Genentech Hillsboro Fill Finish Facility in Hillsboro, Oregon.

You may label your product with the proprietary name, Kadcyla, and will market it as a lyophilized product in two single-use presentations of 100 mg per 15 mL vial and 160 mg per 20 mL vial.

Trastuzumab intermediate will be manufactured at Genentech, Inc., Vacaville, CA and Roche Singapore Technical Operations Pte. Ltd, Singapore. DM1 intermediate will be manufactured at [redacted] (b)(4).

DATING PERIOD

The dating period for ado-trastuzumab emtansine drug product (160 mg/vial) shall be 36 months from the date of manufacture when stored at 2°C to 8°C. The dating period for ado-trastuzumab emtansine drug product (100 mg/vial) shall be 24 months from the date of manufacture when stored at 2°C to 8°C. The date of manufacture shall be defined as the date of [redacted] (b)(4) the formulated drug product. The dating period for your trastuzumab intermediate shall be [redacted] (b)(4). The dating period for your ado-trastuzumab emtansine drug substance shall be [redacted] (b)(4).

We have approved the stability protocols in your license application for the purpose of extending the expiration dating period of the drug substance and drug product under 21 CFR 601.12. Data supporting extension of the expiration dating period should be submitted to the BLA Annual Report.

Consistent with 21 CFR 601.12, Genentech must inform FDA about each change in the product, production process, quality controls, equipment, facilities, responsible personnel, or labeling established in the approved application.

FDA LOT RELEASE

You are not currently required to submit samples of future lots of ado-trastuzumab emtansine 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 Kadcyla (ado-trastuzumab emtansine), 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.

APPROVAL & LABELING

We have completed our review of this application, as amended. It is approved, effective on the date of this letter, for use as recommended in the enclosed agreed-upon labeling text.

We note that your February 13, 2013, submission includes final printed labeling (FPL) for your package insert. We have not reviewed this FPL. You are responsible for assuring that the wording in this printed labeling is identical to that of the approved content of labeling in the structured product labeling (SPL) format.

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. Content of labeling must be identical to the enclosed labeling (text for the package insert, text for the patient 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.

The SPL will be accessible via publicly available labeling repositories.

In addition, within 14 days of the date of this letter, amend any pending supplement that includes labeling changes for this BLA with content of labeling in SPL format to include the changes approved in this supplement.

CARTON AND IMMEDIATE CONTAINER LABELS

We acknowledge your January 30, 2013, submission containing final printed carton and container labels.

ADVISORY COMMITTEE

Your application for Kadcyla (ado-trastuzumab emtansine) 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.

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(s) in pediatric patients unless this requirement is waived, deferred, or inapplicable.

We are waiving the pediatric study requirement for this application because necessary studies are impossible or highly impracticable. Breast cancer is on the list of conditions that do not occur in pediatric patients and qualify for a full waiver.

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 assess signals of serious risks of embryo-fetal toxicity and of increased toxicity due to a variable antibody drug ratio and to identify unexpected serious risks of increased toxicity due to (b) (4)

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA will not be sufficient to assess this serious risk.

Therefore, based on appropriate scientific data, FDA has determined that you are required to conduct the following:

1. Establish a Pregnancy Registry to collect and analyze information for 10 years on pregnancy complications and birth outcomes in women with breast cancer exposed to ado-trastuzumab-emtansine within 6 months of conception or during pregnancy. Submit yearly interim reports, which may be included in your annual reports, on the cumulative findings and analyses from the Pregnancy Registry.

   The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Draft Protocol Submission:	03/13
Final Protocol Submission:	05/13
Interim Report #1:	05/14
Interim Report #2:	05/15
Interim Report #3:	05/16
Interim Report #4:	05/17
Interim Report #5:	05/18
Interim Report #6:	05/19
Interim Report #7:	05/20
Interim Report #8:	05/21
Interim Report #9:	05/22
Study Completion:	05/23
Final Report Submission:	05/24

2. Perform a multivariate characterization study to support the implementation [redacted] of *trans*-succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) [redacted] during manufacture of T-DM1.

   The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	03/13
Study Completion:	05/13
Final Report Submission:	06/13

3. Develop and validate an iCIEF method to use as a drug substance and drug product regulatory method for monitoring the unconjugated antibody content and propose a specification limit for the unconjugated antibody content based on clinical and commercial batch data.

   The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	05/13
Study Completion:	11/13
Final Report Submission:	12/13

4. Provide quarterly reports on the status of any [redacted]. These reports should include, at a minimum, a summary of the root cause analyses, associated corrective actions, and disposition of all affected DM1 batches. Also, provide the disposition of any potentially affected finished product batches using these affected DM1 batches. Submit an interim report documenting that the manufacturing processes have been appropriately controlled at the manufacturing facilities according to Genentech's evaluation. The interim report should include a request for follow-up inspection(s). Submit a final report with a statement concerning the follow-up performed on the [redacted] issues during the course of the FDA inspection(s), an update on whether there have been any further instances of [redacted], and a proposal to prevent [redacted] managed by each site's quality system.

   The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Quarterly Report #1:	05/13
Quarterly Report #2:	08/13
Quarterly Report #3:	11/13
Quarterly Report #4:	02/14
Interim Report:	04/14

Quarterly Report #5: 05/14
Quarterly Report #6: 08/14
Quarterly Report #7: 11/14
Quarterly Report #8: 02/15
Final Report Submission: 04/15

Finally, we have determined that only a clinical trial (rather than a nonclinical or observational study) will be sufficient to assess a signal of a serious risk of increased toxicity in patients with hepatic impairment.

Therefore, based on appropriate scientific data, FDA has determined that you are required to conduct the following:

5. Conduct a clinical trial to evaluate the impact of hepatic impairment on the pharmacokinetics of Kadcyla (ado-trastuzumab emtansine), total trastuzumab, and DM1-containing catabolites. Based on the results of this trial, update the approved Kadcyla labeling with recommendations for appropriate use of Kadcyla in patients with hepatic impairment.

   The timetable you submitted on February 15, 2013, states that you will conduct this trial according to the following schedule:

   Trial Completion: 06/14
   Final Report Submission: 06/15

Submit the protocol(s) to your IND 071072, with a cross-reference letter to this BLA. Submit all final report(s) 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 NOT SUBJECT TO THE REPORTING REQUIREMENTS UNDER SECTION 506B

We remind you of your postmarketing commitments:

6. Transfer the methodology for validated dye ingress testing developed by Genentech to (b)(4). Conduct a study to confirm filling and crimping conditions for container closure integrity using the validated transferred dye ingress method and provide a final report in the 2014 annual report.

   The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Study Completion:	03/13
Final Report Submission:	04/14

7. Conduct a study to assess the risk of endotoxin masking (b)(4) using endotoxin spiked ado-trastuzumab emtansine drug product (b)(4). Submit a final report that includes updated specifications as a Prior Approval Supplement.

   The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Final Report Submission:	03/13

8. If endotoxin masking is observed in the drug product (b)(4), develop an alternative method to quantitate endotoxin in the finished ado-trastuzumab emtansine drug product (b)(4) using routine production conditions. Submit a final report on any changes in the analytical methods as a Prior Approval Supplement.

   The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	09/13
Final Report Submission:	12/13

9. Dedicate (b)(4) for ado-trastuzumab emtansine drug product manufacture and submit a final report of the results from sterilization validation and 3 media fill simulations as a Changes Being Effected Supplement (CBE-0).

   The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Final Report Submission:	06/13

10. Conduct cleaning verification [redacted] until use of [redacted] is implemented and report the updated [redacted] procedures in the 2014 Annual Report.

    The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

    Study completion: 06/13
    Final Report Submission: 04/14

11. Conduct endotoxin spiking and recovery studies [redacted] Submit the final report as a Changes Being Effected in 30 days Supplement (CBE-30).

    The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

    Final Report Submission: 05/13

12. Develop a validated, sensitive, and accurate assay for the detection of neutralizing antibodies to ado-trastuzumab emtansine, including procedures for accurate detection of neutralizing antibodies to ado-trastuzumab emtansine in the presence of ado-trastuzumab emtansine levels that are expected to be present in the serum or plasma at the time of patient sampling. The assay final report will be submitted as a Prior Approval Supplement by June, 2015.

    The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

    Final Report Submission (Assay and Methodology) Date: 06/15

13. Reassess release and stability specifications for ado-trastuzumab emtansine drug substance and drug product through the end of February 2015. Submit the final report as a Changes Being Effected-30 Supplement (CBE-30).

    The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

    Final Report Submission: 05/15

14. Provide a material compatibility assessment [redacted]

[redacted] Provide a toxicological risk assessment [redacted] If significant [redacted] are identified during these assessments, initiate action to mitigate the source(s) of risk to product quality.

The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Material Compatibility Assessment Completion:	04/13
[redacted] Assessment and Toxicological Risk Assessment:	05/13
Final Report Submission:	06/13

15. Conduct ado-trastuzumab emtansine exposure-response analyses for progression-free survival, final overall survival, and safety utilizing data from trial BO25734/TDM4997 (TH3RESA). The results of the exposure-response analyses from both TH3RESA and BO21977/TDM4370g (EMILIA) will be used to determine whether a postmarketing trial is needed to optimize the dose in patients with metastatic breast cancer who have lower exposure to ado-trastuzumab emtansine conjugate at the approved dose (3.6 mg/kg q3w). Submit a final report of the exposure-response analyses based on TH3RESA and EMILIA.

    The timetable you submitted on February 15, 2013, states that you will conduct this study according to the following schedule:

Trial Completion:	06/16
Final Report Submission:	12/16

Submit clinical protocols to your IND 071072 for this product. Submit nonclinical and chemistry, manufacturing, and controls protocols and all postmarketing 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**."

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
Office of Prescription Drug Promotion
5901-B Ammendale Road
Beltsville, MD 20705-1266

As required under 21 CFR 601.12(f)(4), 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 Office of Prescription Drug Promotion (OPDP), see http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm.

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.

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

MEDWATCH-TO-MANUFACTURER PROGRAM

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.

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 Lisa Skarupa, Regulatory Project Manager, at (301) 796-2219.

Sincerely,

{See appended electronic signature page}

Richard Pazdur, M.D.
Director
Office of Hematology and Oncology Products
Center for Drug Evaluation and Research

ENCLOSURE(S):
Content of Labeling
Carton and Container Labeling

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RICHARD PAZDUR
02/22/2013

Attachment D

U.S. Patent No. 7,097,840

US007097840B2

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

(10) **Patent No.: US 7,097,840 B2**
(45) **Date of Patent: Aug. 29, 2006**

(54) **METHODS OF TREATMENT USING ANTI-ERBB ANTIBODY-MAYTANSINOID CONJUGATES**

(75) Inventors: **Sharon Erickson**, Hillsborough, CA (US); **Ralph Schwall**, Pacifica, CA (US); **Mark Sliwkowski**, San Carlos, CA (US); **Walter Blattler**, Brookline, MA (US)

(73) Assignees: **Genentech, Inc.**, South San Francisco, CA (US); **Immunogen, Inc.**, Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 682 days.

(21) Appl. No.: **09/811,123**

(22) Filed: **Mar. 16, 2001**

(65) **Prior Publication Data**

US 2002/0001587 A1 Jan. 3, 2002

Related U.S. Application Data

(60) Provisional application No. 60/238,327, filed on Oct. 5, 2000, provisional application No. 60/327,563, filed on Jun. 23, 2000, provisional application No. 60/189,844, filed on Mar. 16, 2000.

(51) **Int. Cl.**
A61K 39/40 (2006.01)

(52) **U.S. Cl.** **424/178.1**; 424/130.1; 424/135.1; 424/141.1; 424/155.1; 424/158.1

(58) **Field of Classification Search** 424/178.1, 424/179.1, 181.1, 182.1, 130.1, 133.1, 134.1, 424/138.1, 141.1, 143.1, 152.1, 155.1, 156.1, 424/172.1, 174.1
See application file for complete search history.

(56) **References Cited**

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(57) **ABSTRACT**

The application concerns methods of treatment using anti-ErbB receptor antibody-maytansinoid conjugates, and articles of manufacture suitable for use in such methods. In particular, the invention concerns ErbB receptor-directed cancer therapies, using anti-ErbB receptor antibody-maytansinoid conjugates.

44 Claims, 46 Drawing Sheets

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```
VARIABLE HEAVY DOMAIN

                10        20        30        40
2C4     EVQLQQSGPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS
          **  **  *  * *** *                    * *
574     EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA
                                    ** * *
hum III EVQLVESGGGLVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                50 a      60        70        80
2C4     HGKSLEWIG [DVNPNSGGSIYNQRFKG] KASLTVDRSSRIVYM
          *  *  **                    *** *   **** *
574     PGKGLEWVA [DVNPNSGGSIYNQRFKG] RFTLSVDRSKNTLYL
                  ****** *** ****       * * *
hum III PGKGLEWVA [VISGDGGSTYYADSVKG] RFTISRDNSKNTLYL

                abc       90        100ab     110
2C4     ELRSLTFEDTAVYYCAR [NLGPSFYFDY] WGQGTTLTVSS
          ***  **                      **
574     QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS
                           ********
hum III QMNSLRAEDTAVYYCAR [GRVGYSLYDY] WGQGTLVTVSS
```

FIG. 1

Variable Light Domain

```
                10        20        30        40
2C4     DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIGVA] WYQQRP
          **  ****  *       *                 *
574     DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA] WYQQKP
                                  *   **  ***
hum KI  DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA] WYQQKP

                50        60        70        80
2C4     GQSPKLLIY [SASYRYT] GVPDRFTGSGSGTDFTFTISSVQA
          **                  *  *      *    * * *
574     GKAPKLLIY [SASYRYT] GVPSRFSGSGSGTDFTLTISSLQP
                    * *****
hum KI  GKAPKLLIY [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP

                90       100
2C4     EDLAVYYC [QQYYIYPYT] FGGGTKLEIKRT
           * *                 *   *
574     EDFATYYC [QQYYIYPYT] FGQGTKVEIKRT
                    *** *
hum KI  EDFATYYC [QQYNSLPWT] FGQGTKVEIKRT
```

FIG. 2

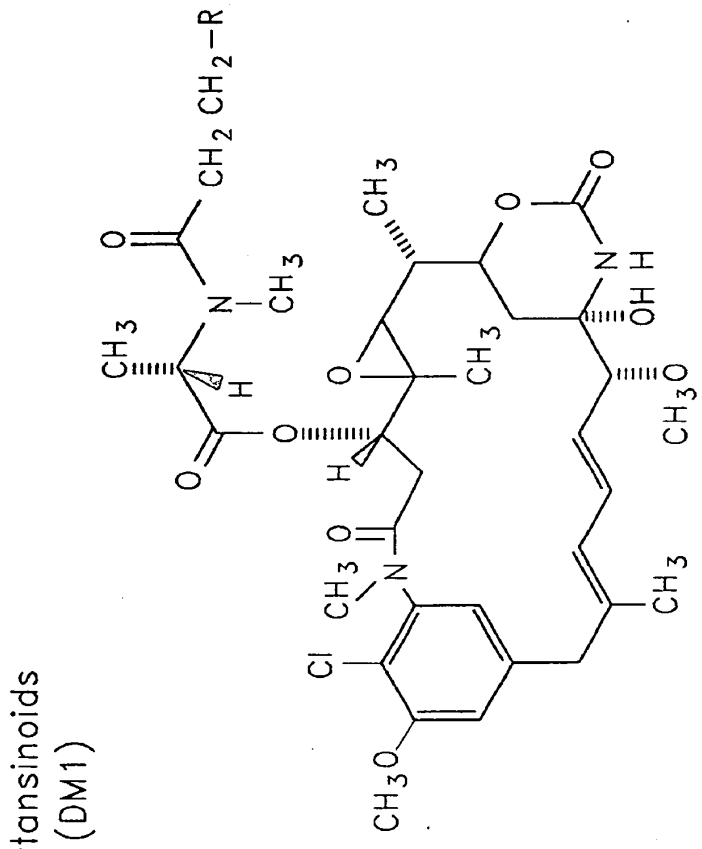
Maytansinoids
(DM1)
CH2 CH2 R
CH3
CH3
CH3
CH3
OH H
CH3O
CH3
CH3
Cl
CH3O

FIG. 3

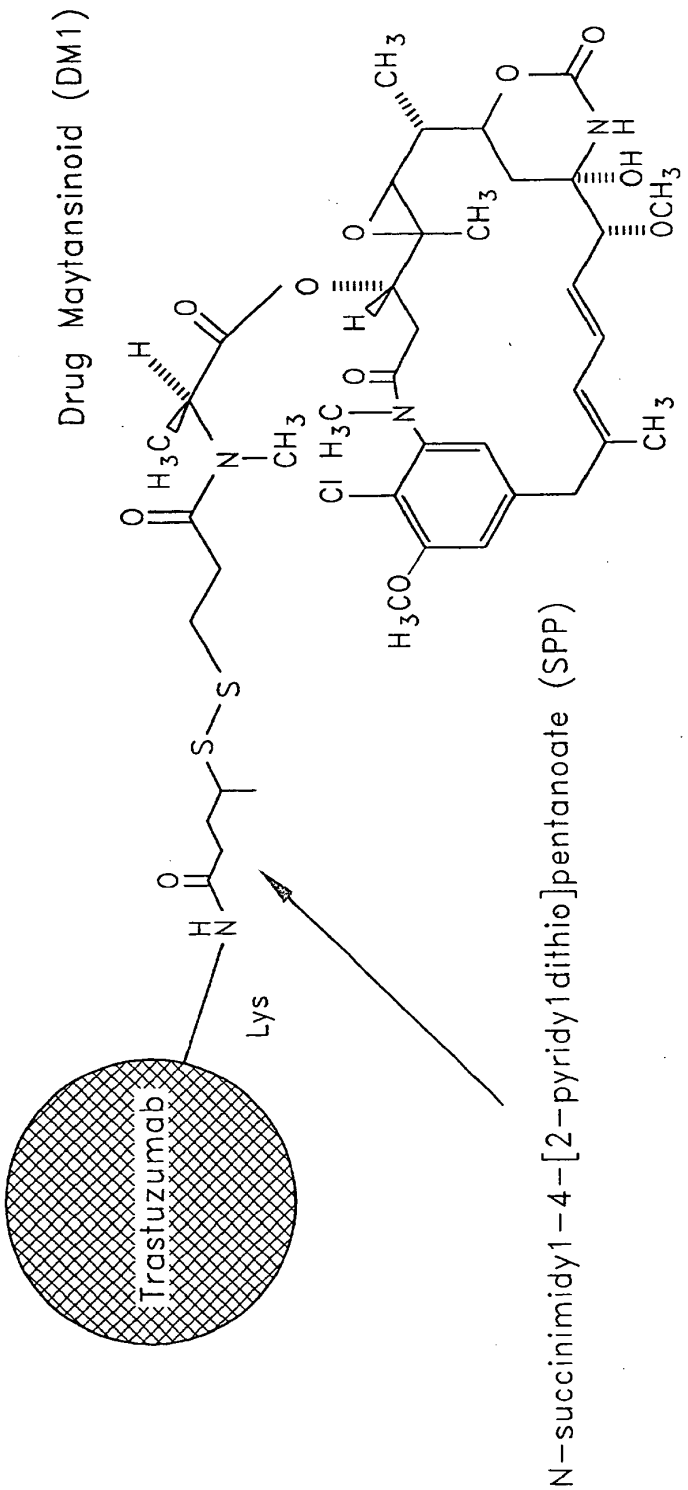
Drug Maytansinoid (DM1)
Trastuzumab
Lys
N-succinimidyl-4-[2-pyridyldithio]pentanoate (SPP)

FIG. 4

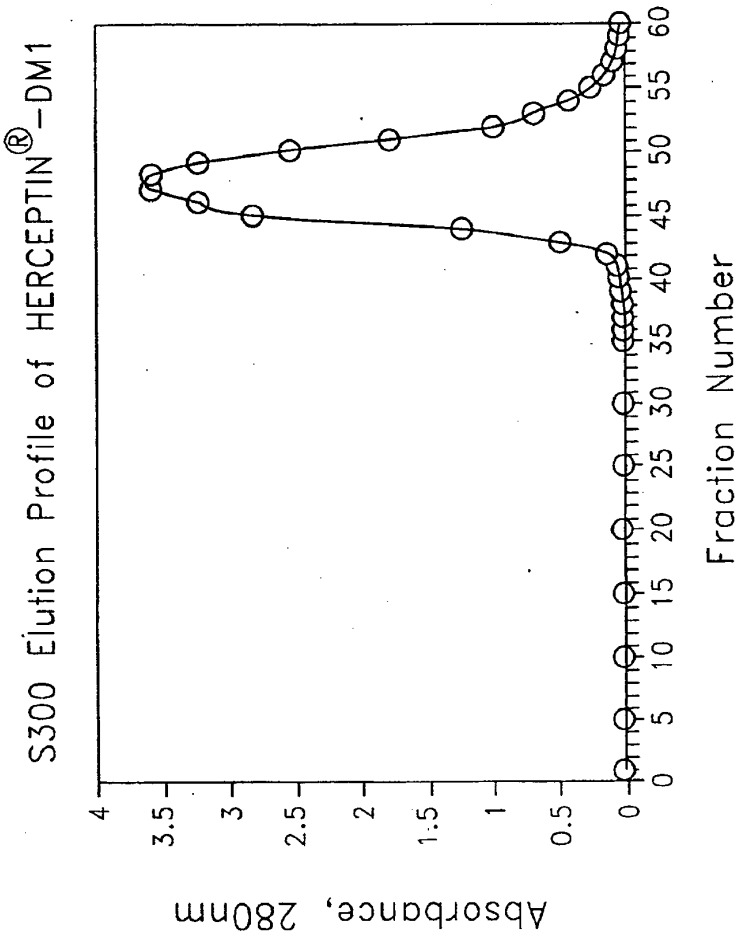
S300 Elution Profile of HERCEPTIN®-DM1
Absorbance, 280nm
Fraction Number
0
0.5
1
1.5
2
2.5
3
3.5
4
0 5 10 15 20 25 30 35 40 45 50 55 60

FIG. 5

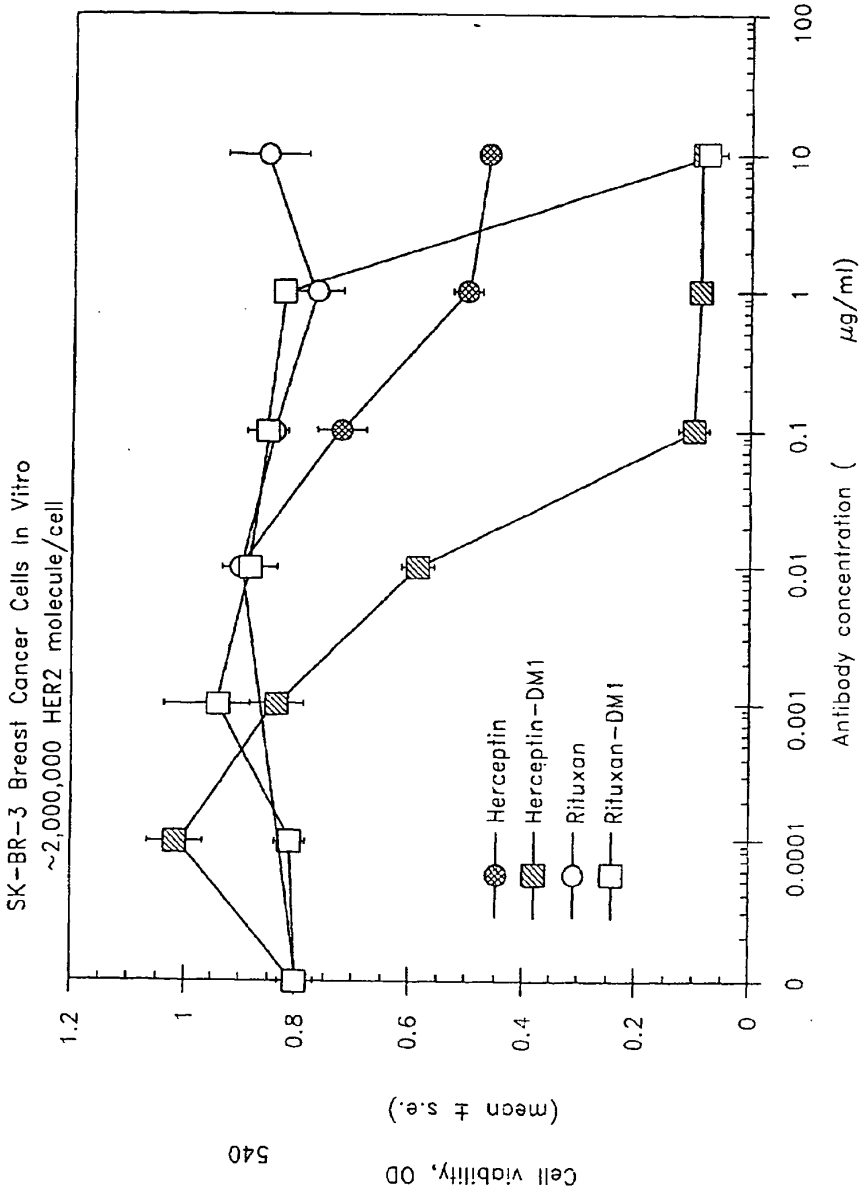
SK-BR-3 Breast Cancer Cells In Vitro
~2,000,000 HER2 molecule/cell
Herceptin
Herceptin-DM1
Rituxan
Rituxan-DM1
Cell viability, OD
540
(mean ± s.e.)
Antibody concentration (μg/ml)
0
0.0001
0.001
0.01
0.1
1
10
100
0.2
0.4
0.6
0.8
1
1.2

FIG. 6

rmaI
hgiAI/aspHI sau3AI maeI
sau3AI mboI/ndeII
mboI/ndeII tru9I dpnII nheI nlaIII tseI rmaI
dpnII bsp1286 dpnI cac8I tseI sphI fnu4HI/bsoFI maeI
pvuI/bspCI mseI nlaIII thaI cac8I nspHI bbvI bfaI
mcrI bsiHKAI tsp509I fnuDII/mvnI cac8I scfI styI
bsiEI bmyI aseI/asnI/vspI bstUI bfaI aluI nspI pstI bsaJI
taqI apaLI/snoI rcaI bsh1236I fnu4HI/bsoFI cac8I blnI
aluI dpnI alw44I/snoI bspHI nruI aluI bbvI cac8I bsgI avaI avrII
1 AAGCTCGATC GGTGCACATT AATTCATGAT CGCGAGCTAG CAGCTTGCAT GCCTGCAGCA GAAATGGTTG AACTCCCGAG AGTGTCCTAC ACCTAGGGGA
TTCGAGCTAG CCACGTGTAA TTAAGTACTA GCGCTCGATC GTCGAACGTA CGGACGTCGT CTTTACCAAC TTGAGGGCTC TCACAGGATG TGGATCCCCT
^start of linker 1 ^end of linker 1 ^start of MMTV promoter
hgiJII
bsp1286
hinPI bmyI styI
hhaI/cfoI banII mwoI
mstI tseI
styI bcgI aviII/fspI fokI fnu4HI/bsoFI
bsaJI ahdI/eam1105I bstF5I bbvI
101 GAAGCAGCCA AGGGGTTGTT TCCCACCAAG GACGACCCGT CTGCGCACAA ACGGATGAGC CCATCAGACA AAGACATATT CATTCTCTGC TGCAAACTTG
CTTCGTCGGT TCCCCAACAA AGGGTGGTTC CTGCTGGGCA GACGCGTGTT TGCCTACTCG GGTAGTCTGT TTCTGTATAA GTAAGAGACG ACGTTTGAAC
mwoI
cac8I hgiJII
hgiAI/aspHI mboII
bsp1286 bsp1286 earI/ksp632I
mwoI bsiHKAI bmyI pleI tru9I sapI mamI
acl1 bmyI banII hphI hinfI mseI aluI bsaBI
aluI
201 GCATAGCTCT GCTTTGCTGG GGCATTGGGG GAAGTTGCGG TTCGTGCTCG CAGGGCTCTC ACCCTTGACT CTTTTAATAG CTCTTCTGTG CAAGATTACA
CGTATCGAGA CGAAACGACC CCGTAACCCC CTTCAACGCC AAGCACGAGC GTCCCGAGAG TGGGAACTGA GAAAATTATC GAGAAGACAC GTTCTAATGT

FIG. 7A

```
                                                                                  claI/bsp106
                                        mnlI    sau96I                            bspDI
                                       ddeI     avaII                            sfaNI
                                      eco81I    asuI                            aciI taqI
        tsp509I       taqI    mnlI    bsu36I/mstII/sauI          mnlI          fnu4HI/bsoFI       eco57I
301 ATCTAAACAA TTCGGAGAAC TCGACCTTCC TCTCCTGAGG CAAGGACCAC AGCCAACTTC CTCTTACAAG CCGCATCGAT TTTGTCCTTC AGAAATAGAA
    TAGATTTGTT AAGCCTCTTG AGCTGGAAGG AGAGGACTCC GTTCCTGGTG TCGGTTGAAG GAGAATGTTC GGCGTAGCTA AAACAGGAAG TCTTTATCTT

                                                                                                      rsaI
        cac8I                                                                    tru9I    tfiI        csp6I
     bsmI          tsp509I                                            maeIII     mseI     hinfI      scaI
401 ATAAGAATGC TTGCTAAAAA TTATATTTTT ACCAATAAGA CCAATCCAAT AGGTAGATTA TTAGTTACTA TGTTAAGAAA TGAATCATTA TCTTTTAGTA
    TATTCTTACG AACGATTTTT AATATAAAAA TGGTTATTCT GGTTAGGTTA TCCATCTAAT AATCAATGAT ACAATTCTTT ACTTAGTAAT AGAAAATCAT

                                                      hgaI
                                                    esp3I            tsp509I                              haeIII/p
          tsp509I                                   bsmBI           munI/mfeI                             stuI
         apoI                                       bsmAI        mnlI    mboII                            haeI
501 CTATTTTTAC TCAAATTCAG AAGTTAGAAA TGGGAATAGA AAATAGAAAG AGACGCTCAA CCTCAATTGA AGAACAGGTG CAAGGACTAT TGACCACAGG
    GATAAAAATG AGTTTAAGTC TTCAATCTTT ACCCTTATCT TTTATCTTTC TCTGCGAGTT GGAGTTAACT TCTTGTCCAC GTTCCTGATA ACTGGTGTCC

                                                                    bsmFI
                                                                  scrFI
                                                                  mvaI           sau96I
                                                                  ecoRII         avaII
                                                                  dsaV           asuI
                                                                  bstNI         ppuMI
 rmaI                                                             bssKI         nlaIV
 maeI                                                             bsaJI         eco0109I/draII
 bfaI                                                bsmAI        apyI          bsmFI      scfI
601 CCTAGAAGTA AAAAAGGGAA AAAAGAGTGT TTTTGTCAAA ATAGGAGACA GGTGGTGGCA ACCAGGGACT TATAGGGGAC CTTACATCTA CAGACCAACA
    GGATCTTCAT TTTTTCCCTT TTTTCTCACA AAAACAGTTT TATCCTCTGT CCACCACCGT TGGTCCCTGA ATATCCCCTG GAATGTAGAT GTCTGGTTGT

                                                                                            mnlI
                                                                                          sau3AI
                                                                                          mboI/ndeII
                                                                                          dpnII
                                                                                          dpnI
                                        tsp509I                                           alwI
                                      tru9I                                               bstYI/xhoII
  sfaNI       bslI       mboII        mseI                   maeIII
701 GATGCCCCCT TACCATATAC AGGAAGATAT GACTTAAATT GGGATAGGTG GGTTACAGTC AATGGCTATA AAGTGTTATA TAGATCCCTC CCTTTTCGTG
    CTACGGGGGA ATGGTATATG TCCTTCTATA CTGAATTTAA CCCTATCCAC CCAATGTCAG TTACCGATAT TTCACAATAT ATCTAGGGAG GGAAAAGCAC
```

FIG. 7B

```
                  rmaI
                  maeI      styI                                                     nlaIII          rmaI
     pleI         bfaI      bsaJI          smlI                                 rsaI                 maeI
     hinfI     aluI      mnlI           bsmAI    mboII          nlaIII          csp6I                bfaI      bsmI
801 AAAGACTCGC CAGAGCTAGA CCTCCTTGGT GTATGTTGTC TCAAGAAGAA AAAGACGACA TGAAACAACA GGTACATGAT TATATTTATC TAGGAACAGG
    TTTCTGAGCG GTCTCGATCT GGAGGAACCA CATACAACAG AGTTCTTCTT TTTCTGCTGT ACTTTGTTGT CCATGTACTA ATATAAATAG ATCCTTGTCC

                                                tspRI
                                 styI           alwNI
                                 bslI      bsmFI
                             bslI bsaJI mnlI    alw26I/bsmAI                                        nlaIII
901 AATGCACTTT TGGGGAAAGA TTTTCCATAC CAAGGAGGGG ACAGTGGCTG GACTAATAGA ACATTATTCT GCAAAAACTT ATGGCATGAG TTATTATGAA
    TTACGTGAAA ACCCCTTTCT AAAAGGTATG GTTCCTCCCC TGTCACCGAC CTGATTATCT TGTAATAAGA CGTTTTTGAA TACCGTACTC AATAATACTT

                                       tru9I
              sau96I             styI  mseI                                          fokI
              haeIII/palI  nlaIV      smlI                          tru9I           bstF5I
              asuI       aciI    bsaJI aflII/bfrI       maeIII       mseI       mnlI       bsmAI
001 TAGCCTTTAT TGGCCCAACC TTGCGGTTCC CAAGGCTTAA GTAAGTTTTT GGTTACAAAC TGTTCTTAAA ACGAGGATGT GAGACAAGTG CTTTGGTGAC
    ATCGGAAATA ACCGGGTTGG AACGCCAAGG GTTCCGAATT CATTCAAAAA CCAATGTTTG ACAAGAATTT TGCTCCTACA CTCTGTTCAC CAAAGGACTG

                               sstI
                               sacI
                               hgiJII
                               hgiAI/aspHI
                               ecl136II
                               bsp1286
                               bsiHKAI
                               bmyI
                             ddeI
                          sau3AI
                          mboI/ndeII
                          dpnII aluI                                      tsp509I
                          dpnI banII ddeI                                apoI
101 TTGGTTTGGT ATCAAAGGTT CTGATCTGAG CTCTGAGTGT TCTATTTTCC TATGTTCTTT TGGAATTTAT CCAAATCTTA TGTAAATGCT TATGTAAACC
    AACCAAACCA TAGTTTCCAA GACTAGACTC GAGACTCACA AGATAAAAGG ATACAAGAAA ACCTTAAATA GGTTTAGAAT ACATTTACGA ATACATTTGG
```

FIG. 7C

bsmAI
esp3I
fokI bsmBI bsrBI tsp45I
bstF5I aciI maeIII
mnlI
hphI
201 AAGATATAAA AGAGTGCTGA TTTTTTGAGT AAACTTGCAA CAGTCCTAAC ATTCACCTCT TGTGTGTTTG TGTCTGTTCG CCATCCCGTC TCCGCTCGTC
TTCTATATTT TCTCACGACT AAAAAACTCA TTTGAACGTT GTCAGGATTG TAAGTGGAGA ACACACAAAC ACAGACAAGC GGTAGGGCAG AGGCGAGCAG
fnu4HI/bsoFI
haeIII/palI
mcrI
eagI/xmaIII/eclXI
eaeI
tfiI bcgI
hinfI
tha1 claI/bsp106 notI
fnu4HI/bsoFI scfI
aluI fnuDII/mvnI
bstUI taqI cac8I cfrI tru9I
nheI aluI aciI aciI pstI
cac8I bsh1236I
nruI bspDI hindIII bsiEI mseI bsgI mnlI
sau3AI
alwI
sau96I
nlaIV
avaII
asuI
sanDI
ppuMI
nlaIV
ecoO109I/draII
mnlI bsmFI aciI
mspI cac8I
hpaII rmaI
scrFI maeI
nciI mboI/ndeII
dsaV dpnII
cauII
bssKI
bsaJI dpnI bfaI
301 ACTTATCCTT CACTTTCCAG AGGGTCCCCC CGCAGACCCC GGATCGCTAG CTCGCGAATC GATAAGCTTG CGGCCGCTTA ACTGCAGAAG TTGGTCGTGA
TGAATAGGAA GTGAAAGGTC TCCCAGGGGG GCGTCTGGGG CCTAGCGATC GAGCGCTTAG CTATTCGAAC GCCGGCGAAT TGACGTCTTC AACCAGCACT
^start of BS intron insert at ClaI
^bp820 in pCI
^start of BS insert at HindIII
pleI
hinfI
mboII
bpuAI
bbsI
nlaIV
hgiCI
banI
bsmAI
taqI
tru9I bsmAI
mseI bsaI
bsrI
tspRI bspMI
maeIII
401 GGCACTGGGC AGGTAAGTAT CAAGGTTACA AGACAGGTTT AAGGAGACCA ATAGAAACTG GGCTTGTCGA GACAGAGAAG ACTCTTGCGT TTCTGATAGG
CCGTGACCCG TCCATTCATA GTTCCAAATG TCTGTCCAAA TTCCTCTGGT TATCTTTGAC CCGAACAGCT CTGTCTCTTC TGAGAACGCA AAGACTATCC
^start of chimeric intron at pCI 857

FIG. 7D

```
                                                                                                fnu4HI/bsoFI
                                                                                             mcrI
                                                                                             eagI/xmaIII/eclXI
                                                                 scrFI                       eaeI
                                                                 mvaI                        cfrI
                                                                 ecoRII                      notI
                                                                 dsaV                        fnu4HI/bsoFI
                                                                 bstNI                   tru9I  haeIII/palI       tsp509I
                                                                 bssKI                   mseI bsiEI   hindIII     ecoRI
                     fokI                                        apyI              aluI smlI aciI aciI aluI      taqI
                     bstF5I                                bslI  bsaJI      tsp509I      aflII/bfrI  cac8I    ecoRV apoI
501  CACCTATTGG TCTTACTGAC ATCCACTTTG CCTTTCTCTC CACAGGTGTC CACTCCCAGG TTCAATTACA GCTCTTAAGC GGCCGCAAGC TTGATATCGA
     GTGGATAACC AGAATGACTG TAGGTGAAAC GGAAAGAGAG GTGTCCACAG GTGAGGGTCC AAGTTAATGT CGAGAATTCG CCGGCGTTCG AACTATAGCT
                                                       ^end of chimeric intron at pCI 989
                                                                                end of BS insert at Hind111^

                                                                          hinPI
              scrFI                                                       hhaI/cfoI
              nciI                                                       thaI
              mspI                                                       fnuDII/mvnI
              hpaII                                                      bstUI
              dsaV                                                       bsh1236I
              cauII                                                     mwoI    sau96I
              bssKI                                                     hinPI mspI
              bsaJI sau3AI                                              hhaI/cfoI
             xmaI/pspAI                                                 cac8I hpaII
             smaI   mboI/ndeII    sau3AI                                bssHII   nlaIV                     mwoI
             scrFI  dpnII         mboI/ndeII                           thaI   scrFI                        hinPI thaI
             nciI   dpnI          dpnII                                fnuDII/mvnI                         hhaI/cfoI
        tseI dsaV   alwI          dpnI                           mnlI  bstUI nciI                         thaI   fnuDII/
        fnu4HI/bsoFI        rmaI  alwI                         xhoI    bsh1236I haeIII/palI               fnuDII/mvnI
        bbvI cauII nlaIV    maeI nlaIV      tsp509I            smlI  hinPI   dsaV                   mwoI   bstUI  bstUI
      scfI   bssKI bstYI/xhoII  bstYI/xhoII                    paeR7I hhaI/cfoI  asuI             tseI     bsh1236I
      pstI   bsaJI bamHI   bfaI bamHI     ecoRI      aluI   avaI    cac8I   cauII                fnu4HI/bsoFI     bsh1236
      bsgI   avaI  alwI   speI  alwI      apoI      hindIII taqI  bssHII  bssKI           mnlI bbvI     aciI   aciI
601 ATTCCTGCAG CCCGGGGGAT CCACTAGTGG ATCCAAAGAA TTCAAAAAGC TTCTCGAGGG CGCGCGCCCG GCCCCCACCC CTCGCAGCAC CCCGCGCCCC
    TAAGGACGTC GGGCCCCCTA GGTGATCACC TAGGTTTCTT AAGTTTTTCG AAGAGCTCCC GCGCGCGGGC CGGGGGTGGG GAGCGTCGTG GGGCGCGGGG
                          ^end of BS intron insert at spe
                                                         ^start of human HER2 from BS at xhol
```

FIG. 7E

```
                   sau96I
                   avaII
                   asuI
                  nlaIV
                 scrFI          nlaIII                           nlaIV                                      hgiAI/aspHI
                 nciI          styI                            hgiJII                           nlaIV     bsp1286
                 mspI          ncoI                            bsp1286                         mspI      bsiHKAI
       mnlI      hpaII         dsaI                        mspA1I/nspBII   mnlI                hpaII     cac8I
      mwoI       dsaV         nlaIV          fnu4HI/bsoFI aciI    bmyI   bseRI              scrFI     thaI
     hinPI       cauII      mspI  bstXI   cac8I  haeIII/palI     banII mnlI                 nciI      fnuDII/mvnI
     hhaI/cfoI   bssKI      hpaII bsaJI  aluI aciI  fnu4HI/bsoFI bseRI      mnlI           dsaV      bstUI  mslI
                                                                                           cauII     bsh1236I
                                                                                           bssKI     aciI bmyI
                                                                                           bsaJI   fnu4HI/bsoFI
1701 GCGCCCTCCC AGCCGGGTCC AGCCGGAGCC ATGGAGCTGG CGGCCTTGTG CCGCTGGGGG CTCCTCCTCG CCCTCTTGCC CCCCGGAGCC GCGAGCACCC
     CGCGGGAGGG TCGGCCCAGG TCGGCCTCGG TACCTCGACC GCCGGAACAC GGCGACCCCC GAGGAGGAGC GGGAGAACGG GGGGCCTCGG CGCTCGTGGG
1                                     M  E  L  A   A  L  C   R  W  G   L  L  L  A   L  L  P   P  G  A   A  S  T  Q

                                                                                                      scrFI
                                                                                                      mvaI
        mspI                                                                                 scrFI    ecoRII
       cfr10I/bsrFI                                                   scrFI                  mvaI     dsaV
    hgiAI/aspHI               fnu4HI/bsoFI                            mvaI                   ecoRII   bstNI
    bsp1286                   aciI                                    ecoRII                 dsaV     bssKI
    bsiHKAI    mslI          tseI                    bsmAI            dsaV                   bstNI    apyI
    bmyI hpaII               fnu4HI/bsoFI            bsaI             bstNI  nlaIII     mnlI bssKI tseI
    apaLI/snoI              aluI                 bsmFI                bssKI nspHI       ecoNI bsaJI fnu4HI/bsoFI
    alw44I/snoI        nlaIII bbvI  nlaIV    bsrI  avaI               apyI  nspI   aciI  bslI apyI  bbvI
1801 AAGTGTGCAC CGGCACAGAC ATGAAGCTGC GGCTCCCTGC CAGTCCCGAG ACCCACCTGG ACATGCTCCG CCACCTCTAC CAGGGCTGCC AGGTGGTGCA
     TTCACACGTG GCCGTGTCTG TACTTCGACG CCGAGGGACG GTCAGGGCTC TGGGTGGACC TGTACGAGGC GGTGGAGATG GTCCCGACGG TCCACCACGT
25    V  C  T   G  T  D   M  K  L  R   L  P  A   S  P  E   T  H  L  D   M  L  R   H  L  Y   Q  G  C  Q   V  V  Q

                                                                          mnlI
       scrFI                                                            scrFI                hgiAI/aspHI
       mvaI                                                             mvaI                 bsp1286
       ecoRII                                                           ecoRII               bsiHKAI
       dsaV                                                   scfI      dsaV                 bmyI
       bstNI                                                  pstI      bstNI               taiI
       bssKI                                                  bsgI      bssKI               maeII
       apyI      hphI    bspMI         cac8I                  sse8387I  apyI               bsaAI
                                                              sbfI   ecoRV
1901 GGGAAACCTG GAACTCACCT ACCTGCCCAC CAATGCCAGC CTGTCCTTCC TGCAGGATAT CCAGGAGGTG CAGGGCTACG TGCTCATCGC TCACAACCAA
     CCCTTTGGAC CTTGAGTGGA TGGACGGGTG GTTACGGTCG GACAGGAAGG ACGTCCTATA GGTCCTCCAC GTCCCGATGC ACGAGTAGCG AGTGTTGGTT
58    G  N  L   E  L  T  Y   L  P  T   N  A  S   L  S  F  L   Q  D  I   Q  E  V   Q  G  Y  V   L  I  A   H  N  Q
```

FIG. 7F

```
                                                                                  haaeIII/palI
                                                                                 eaeI
                  alwNI                                                          cfrI
          bsmFI   alw26I/bsmAI                                                  scrFI
         sau96I   mwoI                                                          mvaI
         nlaIV    bstAPI                                                        ecoRII
         avaII   scfI       mwoI                                                dsaV
         asuI    pstI      tseI            nlaIV                                bstNI
        ppuMI    bsgI      fnu4HI/bsoFI    hgiCI                                bssKI      rmaI
       bspMI   tspRI       bbvI            banI                                 apyI       maeI       bsmAI mspA1I/nspBII
    mnlI eco0109I/draII mnlI  aciI        mnlI      aluI     mnlI              bsaJI       bfaI       bsaI  aciI
001 GTGAGGCAGG TCCCACTGCA GAGGCTGCGG ATTGTGCGAG GCACCCAGCT CTTTGAGGAC AACTATGCCC TGGCCGTGCT AGACAATGGA GACCCGCTGA
    CACTCCGTCC AGGGTGACGT CTCCGACGCC TAACACGCTC CGTGGGTCGA GAAACTCCTG TTGATACGGG ACCGGCACGA TCTGTTACCT CTGGGCGACT
 91 V  R  Q  V   P  L  Q   R  L  R   I  V  R  G   T  Q  L   F  E  D   N  Y  A  L   A  V  L   D  N  G   D  P  L  N

                            scrFI                tseI
                            mvaI                 fnu4HI/bsoFI
                            ecoRII               bbvI
                            dsaV                scfI      taqI
                        mnlI bstNI              pstI      sfuI                sau3AI                     mspA1I/nspB
                      haeIII/palI    cac8I      bsgI      bstBI               mboI/ndeII               sau3AI
             tsp45I  sau96I  bssKI  haeIII/palI tseI      bsiCI               dpnII                    mboI/ndeII
             maeIII  asuI    apyI   stuI       fnu4HI/bsoFI                   dpnI                     dpnII
          ecoNI     nlaIV   bsaJI mnlI    mwoI bbvI     asuII               bstYI/xhoII                dpnI    nlaIV
          bslI      eco0109I/draII haeI   aciI aluI    aluI          mnlI   bglII         mnlI         alwI  aciI
101 ACAATACCAC CCCTGTCACA GGGGCCTCCC CAGGAGGCCT GCGGGAGCTG CAGCTTCGAA GCCTCACAGA GATCTTGAAA GGAGGGGTCT TGATCCAGCG
    TGTTATGGTG GGGACAGTGT CCCCGGAGGG GTCCTCCGGA CGCCCTCGAC GTCGAAGCTT CGGAGTGTCT CTAGAACTTT CCTCCCCAGA ACTAGGTCGC
125  N  T  T   P  V  T   G  A  S  P   G  G  L   R  E  L   Q  L  R  S   L  T  E   I  L  K   G  G  V  L   I  Q  R

                    scrFI
                    mvaI
                    ecoRII
                    dsaV                                                                                     haeIII/
                    bstNI                                                cac8I                       bsrBI   sau96I
                    bssKI                                                aluI                        bslI    asuI
         aluI       apyI                           mboII                pvuII                        aciI  avaI
201 GAACCCCCAG CTCTGCTACC AGGACACGAT TTTGTGGAAG GACATCTTCC ACAAGAACAA mspA1I/nspBII tspRI
                                                                   CCAGCTGGCT CTCACACTGA TAGACACCAA CCGCTCTCGG
    CTTGGGGGTC GAGACGATGG TCCTGTGCTA AAACACCTTC CTGTAGAAGG TGTTCTTGTT GGTCGACCGA GAGTGTGACT ATCTGTGGTT GGCGAGAGCC
158  N  P  Q   L  C  Y  Q   D  T  I   L  W  K   D  I  F  H   K  N  N   Q  L  A   L  T  L  I   D  T  N   R  S  R
```

FIG. 7G

hinPI
hhaI/cfoI
thaI
fnuDII/mvnI
bstUI
mwoI bsh1236I mspI
alwNI hgaI tspRI hpaII mwoI
mnlI alw26I/bsmAI cfr10I/bsrFI
ddeI
sau96I
nlaIV
haeIII/palI
sau96I
pspOMI/bsp120I haeIII/palI
tseI nlaIV haeI
mwoI hgiJII scrFI
mspI eco0109I/draII mvaI
hpaII bsp1286 ecoRII
naeI/ngoMI bmyI dsaV
cfr10I/bsrFI asuI bstNI
cac8I banII bssKI
tseI fnu4HI/bsoFI apyI
fnu4HI/bsoFI asuI mwoI cac8I mnlI
mspAlI/nspBII
bsll
aciI
cac8I
bsp1286
bmyI bbvI
GTGCCCGCTG CAAGGGGCCA CTGCCCACTG ACTGCTGCCA TGAGCAGTGT GCTGCCGGCT GCACGGGCCC CAAGCACTCT GACTGCCTGG CCTGCCTCCA
CACGGGCGAC GTTCCCCGGT GACGGGTGAC TGACGACGGT ACTCGTCACA CGACGGCCGA CGTGCCCGGG GTTCGTGAGA CTGACGGACC GGACGGAGGT
A R C K G P L P T D C C H E Q C A A G C T G P K H S D C L A C L H
225
A C H P C S P M C K G S R C W G E S S E D C Q S L T R T V C A G G C
191
GCCTGCCACC CCTGTTCTCC GATGTGTAAG GGCTCCCGCT GCTGGGGAGA GAGTTCTGAG GATTGTCAGA GCCTGACGCG CACTGTCTGT GCCGGTGGCT
CGGACGGTGG GGACAAGAGG CTACACATTC CCGAGGGCGA CGACCCCTCT CTCAAGACTC CTAACAGTCT CGGACTGCGC GTGACAGACA CGGCCACCGA
301

FIG. 7H

bstEII
scrFI
mvaI
ecoRII
dsaV
bstNI
tseI
fnu4HI/bsoFI
bbvI
tspRI sfaNI
aluI tspRI bslI bsaJI maeIII
2501 CTTCAACCAC AGTGGCATCT GTGAGCTGCA CTGCCCAGCC CTGGTCACCT ACAACACAGA CACGTTTGAG TCCATGCCCA ATCCCGAGGG CCGGTATACA
GAAGTTGGTG TCACCGTAGA CACTCGACGT GACGGGTCGG GACCAGTGGA TGTTGTGTCT GTGCAAACTC AGGTACGGGT TAGGGCTCCC GGCCATATGT
258 F N H S G I C E L H C P A L V T Y N T D T F E S M P N P E G R Y T
cac8I
hinPI
hhaI/cfoI
nlaIV
narI
kasI
hinlI/acyI
hgiCI
haeII aluI
eheI pvuII
banI mspAlI/nspBII
ahaII/bsaHI
ahdI/eaml105I
tsp45I
maeIII
bsmFI
bslI
2601 TTCGGCGCCA GCTGTGTGAC TGCCTGTCCC TACAACTACC TTTCTACGGA CGTGGGATCC TGCACCCTCG TCTGCCCCCT GCACAACCAA GAGGTGACAG
AAGCCGCGGT CGACACACTG ACGGACAGGG ATGTTGATGG AAAGATGCCT GCACCCTAGG ACGTGGGAGC AGACGGGGGA CGTGTTGGTT CTCCACTGTC
291 F G A S C V T A C P Y N Y L S T D V G S C T L V C P L H N Q E V T A
sau3AI
mboI/ndeII
dpnII
dpnI
alwI
nlaIV
bstYI/xhoII
taiI
maeII
bamHI
alwI
mnlI
tsp45I
maeIII
hphI
mnlI
hgiAI/aspHI
bsp1286
bsiHKAI
bmyI
nlaIII
hphI
mnlI
maeIII
bsrI
cac8I
tseI
fnu4HI/bsoFI
bbvI
avaI
bsp1286
bmyI
fokI
bstF5I
mnlI
aciI
mspAlI/nspBII
msl1
2701 CAGAGGATGG AACACAGCGG TGTGAGAAGT GCAGCAAGCC CTGTGCCCGA GTGTGCTATG GTCTGGGCAT GGAGCACTTG CGAGAGGTGA GGGCAGTTAC
GTCTCCTACC TTGTGTCGCC ACACTCTTCA CGTCGTTCGG GACACGGGCT CACACGATAC CAGACCCGTA CCTCGTGAAC GCTCTCCACT CCCGTCAATG
325 E D G T Q R C E K C S K P C A R V C Y G L G M E H L R E V R A V T

FIG. 7I

```
                                                                                                           sau96I
             xcmI                                 sau3AI       scrFI                                       nlaIV
             scrFI                                mboI/ndeII   mvaI                                        avaII
             mvaI                                 dpnII        ecoRII                                      asuI
             ecoRII                               dpnI         dsaV                                       sanDI
             dsaV                tseI            bstYI/xhoII   bstNI                                      ppuMI
             bstNI               fnu4HI/bsoFI                  bssKI                                      nlaIV
             bssKI               bbvI              bglII       apyI                    mspI               eco0109I/draII
   tspRI     apyI           cac8I          mboII          nlaIV                        hpaII aluI         bsmFI     mnlI     tspRI
801 CAGTGCCAAT ATCCAGGAGT TTGCTGGCTG CAAGAAGATC TTTGGGAGCC TGGCATTTCT GCCGGAGAGC TTTGATGGGG ACCCAGCCTC CAACACTGCC
    GTCACGGTTA TAGGTCCTCA AACGACCGAC GTTCTTCTAG AAACCCTCGG ACCGTAAAGA CGGCCTCTCG AAACTACCCC TGGGTCGGAG GTTGTGACGG
358  S  A  N   I  Q  E  F   A  G  C   K  K  I   F  G  S  L   A  F  L   P  E  S   F  D  G  D   P  A  S   N  T  A

                                                                                                        mspI
                                                     sau3AI                                             hpaII
              aluI                                   mboI/ndeII                                       haeIII/palI
    bpmI/gsuI  tseI                   pleI           dpnII                                             eaeI                 ddeI   bpuAI
  bsrBI        fnu4HI/bsoFI           hinfI  mboII dpnI        maeIII                                  cfrI                mnlI   bbsI
  aciI    mwoI bbvI                 bsmAI    earI/ksp632I   bstEII           ddeI  nlaIII        cac8I     drdI   hgaI
901 CCGCTCCAGC CAGAGCAGCT CCAAGTGTTT GAGACTCTGG AAGAGATCAC AGGTTACCTA TACATCTCAG CATGGCCGGA CAGCCTGCCT GACCTCAGCG
    GGCGAGGTCG GTCTCGTCGA GGTTCACAAA CTCTGAGACC TTCTCTAGTG TCCAATGGAT ATGTAGAGTC GTACCGGCCT GTCGGACGGA CTGGAGTCGC
391 P  L  Q  P   E  Q  L   Q  V  F   E  T  L  E   E  I  T   G  Y  L   Y  I  S  A   W  P  D   S  L  P   D  L  S  V

                                                      hinPI
                                                      hhaI/cfoI
                          bsmFI                       nlaIV
                       scrFI                          narI
                       nciI                           kasI
                       mspI                           hinlI/acyI
                       hpaII                          hgiCI
                       dsaV                           haeII                                                           hinPI
        alwNI          cauII     tsp509I              eheI                                              cac8I         hhaI/cfoI
        alw26I/bsmAI   bssKI     ecoRI                banI                                          pvuII          tseI
    mboII    bspMI     bsaJI     apoI                 ahaII/bsaHI mwoI   bslI                       mspA1I/nspBII  fnu4HI/bsoFI
                                                                                              sfaNI aluI  mwoI     bbvI      tspRI
001 TCTTCCAGAA CCTGCAAGTA ATCCGGGGAC GAATTCTGCA CAATGGCGCC TACTCGCTGA CCCTGCAAGG GCTGGGCATC AGCTGGCTGG GGCTGCGCTC
    AGAAGGTCTT GGACGTTCAT TAGGCCCCTG CTTAAGACGT GTTACCGCGG ATGAGCGACT GGGACGTTCC CGACCCGTAG TCGACCGACC CCGACGCGAG
425  F  Q  N   L  Q  V   I  R  G  R   I  L  H   N  G  A   Y  S  L  T   L  Q  G   L  G  I   S  W  L  G   L  R  S
```

FIG. 7J

```
                                                                      sau96I
                                                                      avaII
                                                                      asuI
                                                                     nlaIV
                                                                   scrFI
                                                          draIII   mvaI
                                                         mwoI  bsp1286 bsmFI
                                                         bstAPI bmyI ecoRII
                                                      hgiAI/aspHI  dsaV
                                                      bsp1286 nlaIV bstNI
                        sau96I fokI                   bsiHKAI hgiCI bssKI
                        haeIII/palI                   bmyI    banI  bsaJI
      mnlI              asuI  bstF5I                  apaLI/snoI    apyI
    ddeI  bsrI  tspRI bsrI mnlI                 mnlI  alw44I/snoI  bsaJI    aluI     nlaIV aciI
101 ACTGAGGGAA CTGGGCAGTG GACTGGCCCT CATCCACCAT AACACCCACC TCTGCTTCGT GCACACGGTG CCCTGGGACC AGCTCTTTCG GAACCCGCAC
    TGACTCCCTT GACCCGTCAC CTGACCGGGA GTAGGTGGTA TTGTGGGTGG AGACGAAGCA CGTGTGCCAC GGGACCCTGG TCGAGAAAGC CTTGGGCGTG
458  L  R  E   L  G  S  G   L  A  L   I  H  H   N  T  H  L   C  F  V   H  T  V   P  W  D  Q   L  F  R   N  P  H

                                                                                              sau96I
                                                                                              pspOMI
                                                                                              nlaIV
                                                                                              hgiJII
                                                                                              bsp1286
                                                                                              bmyI
                                                                                              banII
                                                        haeIII/palI                        scrFI
                                                      scrF                                 mvaI
                                                      mvaI                                 ecoRII
                                                      ecoRII                               dsaV
                                                      dsaV                                 bstNI
                                                      bstNI                                bssKI
                            haeIII/palI               bssKI                                bsaJI
                            eaeI                      apyI                 bcgI            bsaJI
                            cfrI                    haeIII/palI           bsaJI            apyI
                           mspI                    sau96I                avaI  bsp1286   sau96I
                           hpaII                   asuI haeI     aluI   hinPI   bmyI     avaII asuI
                           bslI  mnlI             eco0109I/draII pvuII  hhaI/cfoI        asuI  apaI
      aluI      tspRI    cfr10I/bsrFI           mnlI mwoI cac8I mspA1I/nspBII mnlI tspRI nlaIV eco0109I
201 CAAGCTCTGC TCCACACTGC CAACCGGCCA GAGGACGAGT GTGTGGGCGA GGGCCTGGCC TGCCACCAGC TGTGCGCCCG AGGGCACTGC TGGGGTCCAG
    GTTCGAGACG AGGTGTGACG GTTGGCCGGT CTCCTGCTCA CACACCCGCT CCCGGACCGG ACGGTGGTCG ACACGCGGGC TCCCGTGACG ACCCCAGGTC
491  Q  A  L  L  H  T  A   N  R  P   E  D  E  C   V  G  E   G  L  A   C  H  Q  L   C  A  R   G  H  C   W  G  P  G
                                        sequence change from Coussens to Yamamoto^
```

FIG. 7K

scrFI
mvaI
ecoRII
dsaV
bstNI
bssKI
apyI
mwoI
haeIII/palI
sau96I
asuI
nlaIV
hinclI/hindII
bsgI
bsrI
pstI
scfI
bbvI
fnu4HI/bsoFI
tseI
dralII
sau96I tspRI
haeIII/palI
asuI bsrI
301 GGCCCACCCA GTGTGTCAAC TGCAGCCAGT TCCTTCGGGG CCAGGAGTGC GTGGAGGAAT GCCGAGTACT GCAGGGGCTC CCCAGGGAGT ATGTGAATGC
CCGGGTGGGT CACACAGTTG ACGTCGGTCA AGGAAGCCCC GGTCCTCACG CACCTCCTTA CGGCTCATGA CGTCCCCGAG GGGTCCCTCA TACACTTACG
525 P T Q C V N C S Q F L R G Q E C V E E C R V L Q G L P R E Y V N A
mspI
hpaII
tspRI
bsaWI
pflMI
bsp1286
tsp45I
maeIII
sau961
haeIII/palI
tspRI
avaII
bsrI
haeI bmyI bslI
asuI mnlI
bslI
haeI
401 CAGGCACTGT TTGCCGTCGG ACCCTGAGTG TCAGCCCCAG AATGGCTCAG TGACCTGTTT TGGACCGGAG GCTGACCAGT GTGTGGCCTG TGCCCACTAT
GTCCGTGACA AACGGCAGGG TGGGACTCAC AGTCGGGGTC TTACCGAGTC ACTGGACAAA ACCTGGCCTC CGACTGGTCA CACACCGGAC ACGGGTGATA
558 R H C L P C H P E C Q P Q N G S V T C F G P E A D Q C V A C A H Y
tseI
mwoI
fnu4HI/bsoFI
mnlI
sau96I
nlaIV
avaII
asuI
ppuMI
eco0109I/draII mwoI
mspAlI/nspBII
bbvI
aciI
cac8I
sau96I
haeIII/palI
asuI
aciI
mspAlI/nspBII
mnlI
nspHI
nspI
nlaIII
mnlI
bseRI
mwoI
nlaIII
sphI
nspHI
nspI
cac8I
hinPI cac8I
hhaI/cfoI
501 AAGGACCCTC CCTTCTGCGT GGCCCGCTGC CCCAGCGGTG TGAAACCTGA CCTCTCCTAC ATGCCCATCT GGAAGTTTCC AGATGAGGAG GGCGCATGCC
TTCCTGGGAG GGAAGACGCA CCGGGCGACG GGGTCGCCAC ACTTTGGACT GGAGAGGATG TACGGGTAGA CCTTCAAAGG TCTACTCCTC CCGCGTACGG
591 K D P P F C V A R C P S G V K P D L S Y M P I W K F P D E E G A C Q

FIG. 7L

```
                                               scrFI
                                               mvaI
                                               ecoRII
                                               dsaV
                                               bstNI
                                               bssKI
                                               apyI
                                            sau96I                 tseI
                                            avaII  fokI            fnu4HI/bsoFI                                 taiI
                              mslI          asuI   bstF5I          bbvI  aciI          cac8I mnlI  aatII        bsmBI  aciI
3601 AGCCTTGCCC CATCAACTGC ACCCACTCCT GTGTGGACCT GGATGACAAG GGCTGCCCCG CCGAGCAGAG AGCCAGCCCT CTGACGTCCA TCGTCTCTGC
     TCGGAACGGG GTAGTTGACG TGGGTGAGGA CACACCTGGA CCTACTGTTC CCGACGGGGC GGCTCGTCTC TCGGTCGGGA GACTGCAGGT AGCAGAGACG
 625   P  C  P   I  N  C   T  H  S  C   V  D  L   D  D  K   G  C  P  A   E  Q  R   A  S  P   L  T  S  I   V  S  A
```

(tail of the upper annotation column, above aatII/bsmBI: maeII, esp3I, hinlI/acyI bsmAI, ahaII/bsaHI)

```
                                                                                 mspI
                                                      mnlI                       mroI
                                                    sau3AI                       bspMII
                                                    mboI/ndeII                   bspEI
                                                    mamI                         bsaWI
                                                    dpnII                      sau3AI
                                                    dpnI                       mboI/ndeII
                                                    bsaBI                      dpnII
                                                    alwI                       dpnI
                                                   nlaIV                       alwI
                                                   bstYI/xhoII        tseI    bstYI/xhoII          mwoI    tseI
                                                   bamHI              fnu4HI/bsoFI hpaII  rsaI     aciI    fnu4HI/
               bsmI                                alwI       mwoI    bbvI  mboII accIII csp6I sfaNI bsmAI bbvI
3701 GGTGGTTGGC ATTCTGCTGG TCGTGGTCTT GGGGGTGGTC TTTGGGATCC TCATCAAGCG ACGGCAGCAG AAGATCCGGA AGTACACGAT GCGGAGACTG
     CCACCAACCG TAAGACGACC AGCACCAGAA CCCCCACCAG AAACCCTAGG AGTAGTTCGC TGCCGTCGTC TTCTAGGCCT TCATGTGCTA CGCCTCTGAC
 658  V  V  G   I  L  L  V   V  V  L   G  V  V   F  G  I  L   I  K  R   R  Q  Q   K  I  R  K   Y  T  M   R  R  L
```

FIG. 7M

```
                                                                                   sau3AI
                                                                      hinPI        mboI/ndeII
                                                                      hhaI/cfoI    dpnII
                                                                   scrFI          nlaIV
                                                                   mvaI           bstYI/xhoII
                                           rmaI                    ecoRII         bamHI
                                  mspAlI/nspBII                    dsaV         aciI
      scfI                        aciI     maeI                    bstNI     sfaNI dpnI     esp3I     mnlI
      pstI        mwoI           fnu4HI/bsoFI   aciI               bssKI     mamI alwI      bsmBI    ddeI
      bsgI        aluI          nlaIV      bfaI       sfaNI        apyI      bsaBI alwI     bsmAI   aluI      hphI
801 CTGCAGGAAA CGGAGCTGGT GGAGCCGCTG ACACCTAGCG GAGCGATGCC CAACCAGGCG CAGATGCGGA TCCTGAAAGA GACGGAGCTG AGGAAGGTGA
    GACGTCCTTT GCCTCGACCA CCTCGGCGAC TGTGGATCGC CTCGCTACGG GTTGGTCCGC GTCTACGCCT AGGACTTTCT CTGCCTCGAC TCCTTCCACT
691 L  Q  E  T   E  L  V   E  P  L   T  P  S  G   A  M  P   N  Q  A   Q  M  R  I   L  K  E   T  E  L   R  K  V  R

                                                      bslI
                                                    sau3AI
                                                    mboI/ndeII
                                                    dpnII
           sau3AI mwoI                              dpnI
           mboI/ndeII                               alwI                                  mscI/balI
           dpnII  hinPI                            nlaIV                                  haeI
           dpnI   hhaI/cfoI                        bstYI/xhoII                            eaeI
          bstYI/xhoII                              bamHI                               tspRI
          alwI   haeII          accI      sfaNI    alwI                           tsp509I cfrI mslI
                                                                                apoI  bsrI haeIII/palI     mnlI
901 AGGTGCTTGG ATCTGGCGCT TTTGGCACAG TCTACAAGGG CATCTGGATC CCTGATGGGG AGAATGTGAA AATTCCAGTG GCCATCAAAG TGTTGAGGGA
    TCCACGAACC TAGACCGCGA AAACCGTGTC AGATGTTCCC GTAGACCTAG GGACTACCCC TCTTACACTT TTAAGGTCAC CGGTAGTTTC ACAACTCCCT
725  V  L  G   S  G  A   F  G  T  V   Y  K  G   I  W  I   P  D  G  E   N  V  K   I  P  V   A  I  K  V   L  R  E

                                                                   nlaIV
                                                                   hgiJII               mwoI
                                                   taiI            bsp1286             bslI
        fokI                                       maeII           bmyI                aciI
        bstF5I                         ddeI       bsaAI            banII   ndeI  bsmAI   bglI      sfaNI
001 AAACACATCC CCCAAAGCCA ACAAAGAAAT CTTAGACGAA GCATACGTGA TGGCTGGTGT GGGCTCCCCA TATGTCTCCC GCCTTCTGGG CATCTGCCTG
    TTTGTGTAGG GGGTTTCGGT TGTTTCTTTA GAATCTGCTT CGTATGCACT ACCGACCACA CCCGAGGGGT ATACAGAGGG CGGAAGACCC GTAGACGGAC
758  N  T  S   P  K  A  N   K  E  I   L  D  E   A  Y  V  M   A  G  V   G  S  P   Y  V  S  R   L  L  G   I  C  L
```

FIG. 7N

sau96I
scrFI avaII
mvaI asuI
ecoRII ppuMI
aciI dsaV scrFI
thaI bstNI mvaI
scrFI fnuDII/mvnI hgiJII alwNI
nciI bstUI bssKI ecoRII
mspI bsh1236I bsaJI dsaV
hpaII sacII/sstII bsp1286 eco0109I/draII
dsaV mspA1I/nspBII bmyI bstNI
mnlI nlaIII kspI apyI nlaIV alw26I/bsmAI
tseI tth111I/aspI dsaI hin1I/acyI bssKI
fnu4HI/bsoFI bsiI cauII bsaJI hgaI banII apyI
bbvI ddeI pflFI bssKI aciI ahaII/bsaHI bsaJI bspMI
aluI
xcmI pvuII tsp45I
dsaI mspA1I/nspBII
bsaJI tseI maeIII
fokI fnu4HI/bsoFI
bstF5I bbvI hphI aluI
4101 ACATCCACGG TGCAGCTGGT GACACAGCTT ATGCCCTATG GCTGCCTCTT AGACCATGTC CGGGAAAACC GCGGACGCCT GGGCTCCCAG GACCTGCTGA
TGTAGGTGCC ACGTCGACCA CTGTGTCGAA TACGGGATAC CGACGGAGAA TCTGGTACAG GCCCTTTTGG CGCCTGCGGA CCCGAGGGTC CTGGACGACT
791 T S T V Q L V T Q L M P Y G C L L D H V R E N R G R L G S Q D L L N
mnlI
bpmI/gsuI
scrFI
mvaI
ecoR11
dsaV
bstNI fnu4HI/bsoFI
styI fokI aluI bssKI fokI aciI rsaI
bsaJI bstF5I apyI bstF5I csp6I bsmFI cfrI
4201 ACTGGTGTAT GCAGATTGCC AAGGGGATGA GCTACCTGGA GGATGTGCGG CTCGTACACA GGGACTTGGC CGCTCGGAAC GTGCTGGTCA AGAGTCCCAA
TGACCACATA CGTCTAACGG TTCCCCTACT CGATGGACCT CCTACACGCC GAGCATGTGT CCCTGAACCG GCGAGCCTTG CACGACCAGT TCTCAGGGTT
825 W C M Q I A K G M S Y L E D V R L V H R D L A A R N V L V K S P N
eaeI taiI pleI
maeII hinfI
fnu4HI/bsoFI
haeIII/palI
bsrBI
aciI
bsrI
tseI
fnu4HI/bsoFI
mwoI
cac8I
bbvI
bsmAI
csp6I
nlaIII
bstXI
rsaI msII
bsp1286
bmyI
nlaIV xcmI
hgiCI pflMI
banI bslI
hinPI
haeII
fokI hhaI/cfoI
bstF5I
4301 CCATGTCAAA ATTACAGACT TCGGGCTGCT GACATTGACG AGACAGAGTA CCATGCAGAT GGGGGCAAGG TGCCCATCAA GTGGATGGCG
GGTACAGTTT TAATGTCTGA AGCCCGACGA CTGTAACTGC TCTGTCTCAT GGTACGTCTA CCCCCGTTCC ACGGGTAGTT CACCTACCGC
858 H V K I T D F G L A R L L D I D E T E Y H A D G G K V P I K W M A

FIG. 7O

mspI
hpaII
naeI/ngoMI
cfr10I/bsrFI
cac8I
sgrAI
pleI
hinfI
bpmI/gsuI
aciI
aciI hphI
msiI
tsp45I
maeIII
aluI
haeIII/paiI
sau96I
asuI
nlaIV
bsiI
4401 CTGGAGTCCA TTCTCCGCCG GCGGTTCACC CACCAGAGTG ATGTGTGGAG TTATGGTGTG ACTGTGTGGG AGCTGATGAC TTTTGGGGCC AAACCTTACG
GACCTCAGGT AAGAGGCGGC CGCCAAGTGG GTGGTCTCAC TACACACCTC AATACCACAC TGACACACCC TCGACTACTG AAAACCCCGG TTTGGAATGC
891 L E S I L R R R F T H Q S D V W S Y G V T V W E L M T F G A K P Y D
scrFI
nciI
mspI
hpaII
dsaV
cauII
bssKI
xmaI/pspAI
bsiI smaI
sau3AI scrFI
mboI/ndeII
dpnII nciI
dpnI dsaV sau3AI
alwI cauII mboI/ndeII
nlaIV bssKI dpnII
bstYI/xhoII dpnI
bamHI bsaJI alwI bspMI
alwI avaI bstYI/xhoII
tseI
mwoI
fnu4HI/bsoFI
bbvI
fnu4HI/bsoFI
aciI
bsrBI
sau3AI
mboI/ndeII
dpnII
dpnI
bclI
nlaIII
nlaIII
accI msiI
4501 ATGGGATCCC AGCCCGGGAG ATCCCTGACC TGCTGGAAAA GGGGGAGCGG CTGCCCCAGC CCCCCATCTG CACCATTGAT GTCTACATGA TCATGGTCAA
TACCCTAGGG TCGGGCCCTC TAGGGACTGG ACGACCTTTT CCCCCTCGCC GACGGGGTCG GGGGGTAGAC GTGGTAACTA CAGATGTACT AGTACCAGTT
925 G I P A R E I P D L L E K G E R L P Q P P I C T I D V Y M I M V K

FIG. 7P

sau96I
sanDI
scrFI
mvaI nlaIV
ecoRII
dsaV avaII
bstNI
bssKI
bsaJI
apyI asuI
haeIII/palI
mscI/balI
hinPI
haeI ppuMI
hhaI/cfoI
tsp509I
eaeI nlaIV
haeII
bslI
ecoRI
nlaIII eco0109I/draII
fokI
apoI
aciI cfrI bsmFI
afeI/eco47III bstF5I
haeIII/palI
eaeI tfiI
fokI pleI
cfrI hinfI
bstF5I
4601 ATGTTGGATG ATTGACTCTG AATGTCGCCC AAGATTCCGG GAGTTGGTGT CTGAATTCTC CCGCATGGCC AGGGACCCCC AGCGCTTTGT GGTCATCCAG
TACAACCTAC TAACTGAGAC TTACAGCCGG TTCTAAGGCC CTCAACCACA GACTTAAGAG GGCGTACCGG TCCCTGGGGG TCGCGAAACA CCAGTAGGTC
958 C W M I D S E C R P R F R E L V S E F S R M A R D P Q R F V V I Q
scrFI
mvaI
ecoRII
dsaV
bstNI
bssKI
apyI
sexAI
sau96I
avaII
ppuMI
nlaIV sfaNIU
eco0109I/draII mnlI
bsmFI fokI ddeI
bsrBI mnlI aciI tspRI bpmI/gsuI nlaIII asuI bstF5I bseRI
sau96I
haeIII/palI
asuI
sau96I
pspOMI/bsp120I
nlaIV
hgiJII
bsp1286
bmyI
banII bsmFI
asuI bstXI styI
apaI bsrI bsaJI
mnlI
4701 AATGAGGACT TGGGCCCAGC CAGTCCCTTG GACAGCACCT TCTACCGCTC ACTGCTGGAG GACGATGACA TGGGGACCTT GGTGGATGCT GAGGAGTATC
TTACTCCTGA ACCCGGGTCG GTCAGGGAAC CTGTCGTGGA AGATGGCGAG TGACGACCTC CTGCTACTGT ACCCCCTGGA CCACCTACGA CTCCTCATAG
991 N E D L G P A S P L D S T F Y R S L L E D D D M G D L V D A E E Y L

FIG. 7Q

scrFI
nciI
mspI
hpaII
dsaV
cauII
bssKI
bslI
xmaI/pspAI
smaI
scrFI
nciI
dsaV
cauII
bssKI
bsaJI
mwoI
bslI bsaJI hinPI
alwNI bslI avaI hhaI/cfoI
mboII alw26I/bsmAI haeII
4801 TGGTACCCCA GCAGGGCTTC TTCTGTCCAC ACCCTGCCCC GGGCGCTGGG GGCATGGTCC ACCACAGGCA CCGCAGCTCA TCTACCAGGA GTGGCGGTGG
ACCATGGGGT CGTCCCGAAG AAGACAGGTC TGGGACGGGG CCCGCGACCC CCGTACCAGG TGGTGTCCGT GGCGTCGAGT AGATGGTCCT CACCGCCACC
1025 V P Q Q G G F F C P D P A P G A G G G M V H H R H R S S S T R SS G G G
bsmAI
xcmI
scrFI
mvaI
ecoRII
sau96I bsaI
nlaIV dsaV
haeIII/paiI
hgiJII
bsp1286 mnlI asuI bstNI
bmyI bseRI bssKI
sau96I rmaI banII mboII ecoC109I/draII
avaII maeI nlaIV mnlI earI/ksp632I apyI
asuI bfaI bpmI/gsuI eco57I mnlI bsaJI
4901 GGACCTGACA CTAGGGCTGG AGCCCTCTGA AGAGGAGGCC CCCAGGTCTC CACTGGCACC CTCCGAAGGG GCTGGCTCCG ATGTATTTGA TGGTGACCTG
CCTGGACTGT GATCCCGACC TCGGGAGACT TCTCCTCCGG GGGTCCAGAG GTGACCGTGG GAGGCTTCCC CGACCGAGGC TACATAAACT ACCACTGGAC
1058 D L T L G L E P S E E E E A P R S P L A P S E G A G S D V F D G D L
scrFI
mvaI
ecoRII
dsaV
bstNI
bssKI
bslI
bsaJI
tsp45I
maeIII
hphI apyI
bstEII
nlaIV
cac8I
hgiCI
banI mnlI
bsrI bslI
tspRI bslI
nlaIV
hgiCI
bssKI
bseRI
mboII ecoC109I/draII
apyI
bsaJI
CACTGGCACC CTCCGAAGGG GCTGGCTCCG ATGTATTTGA TGGTGACCTG
GTGACCGTGG GAGGCTTCCC CGACCGAGGC TACATAAACT ACCACTGGAC

FIG. 7R

sau96I
nlaIV
avaII
asuI
rsaI
csp6I
mnlI
tspRI ppuMI
scfI aciI
mnlI mspAlI/nspBII
eco0109I/draII
dde I
mnlI bsmAI
AGGACCCCAC AGTACCCCTG
CCCTCTACAG CGGTACAGTG
GCTGCAAAGC CTCCCCACAC
CGACGTTTCG GAGGGGTGTG
TACTGGGGTC GGGAGATGTC
GCCATGTCAC TCCTGGGGTG
TCATGGGGAC GGGAGACTCT
P L Q R Y S E D P T V P L P S E T
sau96I
haeIII/palI
asuI
sau96I
pspOMI/bsp120I
nlaIV
hgiJII
eco0109I/draII
bsp1286
bmyI
banII
sau96I
asuI
nlaIV
apaI
haeIII/palI
avaI eco0109I/draII
asuI
bslI mnlI mnlI
ACCCAGCCAG ATGTTCGGCC
CCAGCCCCCT TCGCCCCGAG
GGTCGGGGGA AGCGGGGCTC
TCCCGGGAGA
Q P P P S P R E G P L
tseI
fnu4HI/bsoFI
bbvi
scfI
pstI
bsgI
bspMI
tailI
maeII
TGAATATGTG
ACTTATACAC
TTGGTCGGTC
TACAAGCCGG
V R P
N Q P D
E Y V
P Q P
L T C S
A T L E R A K T L S P G K N G V V K D V F A F G G A
scrFI
mvaI
ecoRII
dsaV
bstNI
bssKI
bsaJI
apyI
haeIII/palI
sau96I pleI
asuI hinfI
bsaJI mboII
taiI
maeII
dsaI
bsaJI
nlaIV
hgiCI
banI
tseI
fnu4HI/bsoFI
bbvI
cac8I mwoI bspMI
nlaIV
hgiCI
banI
GCCTGCTGCC CGACCTGCTG GTGCCACTCT GGAAAGGGCC AAGACTCTCT CCCCAGGGAA GAATGGGGTC GTCAAAGACG TTTTTGCCTT TGGGGGTGCC
CGGACGACGG GCTGGACGAC CACGGTGAGA CCTTTCCCGG TTCTGAGAGA GGGGTCCCTT CTTACCCCAG CAGTTTCTGC AAAAACGGAA ACCCCCACGG

FIG. 7S

scrFI
mvaI
ecoRII
dsaV
bstNI
bssKi
bsaJI
apyI
csp6I
avaI scaI
bslI
bseRI
mnlI
aluI
mnlI bbvI
ddeI
fnu4HI/bsoFI
tseI
mnlI
bseRI
mnlI
eco57I
taqI
mnlI
bsrI
sau96I
avaII
asuI
nlaIV
bsmFI
191 V E N P E Y L T P Q G G A A P Q P H P P P A F S P A F D N L Y Y W D
301 GTGGAGAACC CCGAGTACTT GACACCCCAG GGAGGAGCTG CCCCTCAGCC CCACCCTCCT CCTGCCTTCA GCCCAGCCTT CGACAACCTC TATTACTGGG
CACCTCTTGG GGCTCATGAA CTGTGGGGTC CCTCCTCGAC GGGGAGTCGG GGTGGGAGGA GGACGGAAGT CGGGTCGGAA GCTGTTGGAG ATAATGACCC
sau96I
nlaIV
avaII
asuI
ppuMI
eco0109I/draII
scrFI
mvaI
ecoRII
dsaV
bstNI
bssKi
apyI
bslI aciI
bsrBI
banII
nlaIV
hgiJII
bsp1286
bmyI
bslI
bsmFI
401 ACCAGGACCC ACCAGAGCGG GGGGCTCCAC CCAGCACCTT CAAAGGGACA CCTACGGCAG AGAACCCAGA GTACCTGGGT CTGCACGTGC CAGTGTGAAC
TGGTCCTGGG TGGTCTCGCC CCCCGAGGTG GGTCGTGGAA GTTTCCCTGT GGATGCCGTC TCTTGGGTCT CATGGACCCA GACCTGCACG GTCACACTTG
225 Q D P P E R G A P P S T F K G T P T A E N P E Y L G L D V P V O

FIG. 7T

```
                                                                                                               rmaI
                                                                                                               maeI
                                                                                                              styI
                                                                                                              bsaJI
                                                                                                              blnI
                                                                                                              avrII
                                                                                                            haeIII/pal
       mwoI                              ddeI                      mnlI   aluI                                stuI
        haeIII/palI                      mnlI                    haeIII/palI                      mnlI        haeI
       haeI       aciI                   eco81I    mwoI        fnu4HI/bsoFI                   mnlI           mnlI bfaI
                                         bsu36I/mstII/sauI     aciI    ddeI                   bseRI
501 CAGAAGGCCA AGTCCGCAGA AGCCCTGATG TGTCCTCAGG GAGCAGGGAA GGCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC
    GTCTTCCGGT TCAGGCGTCT TCGGGACTAC ACAGGAGTCC CTCGTCCCTT CCGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG

                                                                     sau96I
                                                                    sau96I
                                                                    pspOMI/bsp120I
                                                                    nlaIV
                                                                    hgiJII
                                                                thaI haeIII/palI
                                                                fnuDII/mvnI
                                                               sacII/sstII
                                                               mspA1I/nspBII
                                                               kspI bsp1286
                                                               dsaI bmyI
                                                               bsaJI asuI
                                                              fnu4HI/bsoFI
                                                             haeIII/palI
                                                            mcrI    banII
                                 xhoI                       eagI/smaIII/eclXI
                                 smlI                       eaeI cac8I
                               pleI                         cfrI aciI
                             taqI taqI                      bsiEI   asuI
                           salI  paeR7I          rmaI      notI bstUI
                   claI/bsp106  hinfI      rsaI  maeI      fnu4HI/bsoFI                                mnlI
          aluI  bspDI      hincII/hindII  csp6I bfaI      aciI bsh1236I                   taiI        ecoNI
         hindIII taqI     accI  avaI     scaI  xbaI bsrBI aciI apaI      mnlI drdI      maeII       bslI
601 TAGGCTTTTG CAAAAAGCTT ATCGATACCG TCGACTCGAG AGTACTTCTA GAGCGGCCGC GGGCCCATCG CCTCTGACAG CAACGTCTAT GACCTCCTAA
    ATCCGAAAAC GTTTTTCGAA TAGCTATGGC AGCTGAGCTC TCATGAAGAT CTCGCCGGCG CCCGGGTAGC GGAGACTGTC GTTGCAGATA CTGGAGGATT
              ^start of BS insert of HER2 xba-hind111                 ^start of hgh ex 4 (cla/nar)
                                   ^end of human HER2 insert fropm BS at xhol                             ^TG PCR 5' pri
```

FIG. 7U

```
                                                                   mspI
                                                                    hpaII
                                                                   scrFI
      rmaI                                                         nciI
      maeI                                                         dsaV            mboII
      bfaI                                                         cauII          sau3AI
   sau96I                                                          bssKI          mboI/ndeII
   avaII                                                           bslI           dpnII
   asuI              fokI                                     tseI bslI           dpnI
  ppuMI    mnlI      bstF5I                                   fnu4HI/bsoFI       bstYI/xhoII
  eco0109I/draII   sfaNI                   mnlI     mboII     bbvI  bsaJI bsrI   bglII             scfI        taqI
701 AGGACCTAGA GGAAGGCATC CAAACGCTGA TGGGGAGGCT GGAAGATGGC AGCCCCCGGA CTGGGCAGAT CTTCAAGCAG ACCTACAGCA AGTTCGACAC
    TCCTGGATCT CCTTCCGTAG GTTTGCGACT ACCCCTCCGA CCTTCTACCG TCGGGGGCCT GACCCGTCTA GAAGTTCGTC TGGATGTCGT TCAAGCTGTG
                                    ^end of ex 4/ start ex 5

801 AAACTCACAC AACGATGACG CACTACTCAA GAACTACGGG CTGCTCTACT GCTTCAGGAA GGACATGGAC AAGGTCGAGA CATTCCTGCG CATCGTGCAG
    TTTGAGTGTG TTGCTACTGC GTGATGAGTT CTTGATGCCC GACGAGATGA CGAAGTCCTT CCTGTACCTG TTCCAGCTCT GTAAGGACGC GTAGCACGTC
```

FIG. 7V

```
                                        scrFI
                                        nciI
                                        mspI
                                        hpaII
                                        dsaV
                                       xmaI/pspAI
                                       smaI
                                       scrFI
                                       nciI                                     sau96I
                                       dsaV                                     haeIII/palI
                                       cauII                                    asuI
                                       bssKI                                  scrFI scrFI
                     aluI          tseI cauII                                 mvaI   mvaI
                    pvuII          fnu4HI/bsoFI                               ecoRII ecoRII
                   tseI            bbvI  bssKI                                dsaV  dsaV               bsp1286
      bsrBI        mwoI         rmaI  bsaJI    mslI                          bstNI bstNI              bmyI
      bslI         fnu4HI/bsoFI maeI  mwoI     fokI         mnlI             bssKI bssKI           bsrI
      aciI    mnlI mspAII/nspBII  aluI  avaI   bstF5I  tsp45I      tspRI     bslI  apyI          bpmI/gsuI
     fnu4HI/bsoFI  bbvI         bfaI  bglI     sfaNI   maeIII     bsrI  mnlI apyI bsaJI       mslI tspRI
901 TGCCGCTCTG TGGAGGGCAG CTGTGGCTTC TAGCTGCCCG GGTGGCATCC CTGTGACCCC TCCCCAGTGC CTCTCCTGGC CCTGGAAGTT GCCACTCCAG
    ACGGCGAGAC ACCTCCCGTC GACACCGAAG ATCGACGGGC CCACCGTAGG GACACTGGGG AGGGGTCACG GAGAGGACCG GGACCTTCAA CGGTGAGGTC
                                           ^end of spe-sma pBK-CMV/hgh insert to replace intron
                                                                                   ^TG PCR 3' primer

                                                    rmaI
                              tru9I                 maeI
                              mseI                  bfaI
                            tsp509I  sfaNI        ahdI/eam1105I      sspI          mnlI
001 TGCCCACCAG CCTTGTCCTA ATAAAATTAA GTTGCATCAT TTTGTCTGAC TAGGTGTCCT TCTATAATAT TATGGGGTGG AGGGGGGTGG TATGGAGCAA
    ACGGGTGGTC GGAACAGGAT TATTTTAATT CAACGTAGTA AAACAGACTG ATCCACAGGA AGATATTATA ATACCCCACC TCCCCCCACC ATACCTCGTT
                                          ^end of hgh exon5
```

FIG. 7W

```
          bslI
        sau96I
        haeIII/palI
        asuI
      sau96I
      pspOMI/bsp120I
      nlaIV
      hgiJII
      bsp1286
      bmyI
      banII                                 cac8I
      asuI                               haeIII/palI
      apaI           mboII              sau96I                                                                   mnlI
     nlaIV           bpuAI            eco0109I/draII                     bpmI/gsuI                   mwoI     bslI
     eco0109/draII   bbsI        scfI asuI    aciI            nlaIV    aluI        tspRI                tspRI        aciI
101  GGGGCCCAAG TTGGGAAGAC AACCTGTAGG GCCTGCGGGG TCTATTCGGG AACCAAGCTG GAGTGCAGTG GCACAATCTT GGCTCACTGC AATCTCCGCC
     CCCCGGGTTC AACCCTTCTG TTGGACATCC CGGACGCCCC AGATAAGCCC TTGGTTCGAC CTCACGTCAC CGTGTTAGAA CCGAGTGACG TTAGAGGCGG

                                                                        sphI
                                                                        nspHI
                                                                        nspI       scrFI
      scrFI                                                         scrFI          mvaI
      mvaI                                                          mvaI           ecoRII
      ecoRII                                                        ecoRII         dsaV
      dsaV                                                          dsaV     nlaIII    celII/espI
      bstNI                                                         bstNI ppu10I   bstNI
      bssKI         tfiI                                            bssKI nsiI/avaIII  blpI/bpu1102I            esp3I
      bsaJI         hinfI       ddeI     avaI                  tfiI apyI nlaIII    bssKI    aluI                 bsmBI
      apyI         bcgI        mnlI    mnlI                   hinfI    cac8I       apyI ddeI     tsp509I         bsmAI
201  TCCTGGGTTC AAGCGATTCT CCTGCCTCAG CCTCCCGAGT TGTTGGGATT CCAGGCATGC ATGACCAGGC TCAGCTAATT TTTGTTTTTT TGGTAGAGAC
     AGGACCCAAG TTCGCTAAGA GGACGGAGTC GGAGGGCTCA ACAACCCTAA GGTCCGTACG TACTGGTCCG AGTCGATTAA AAACAAAAAA ACCATCTCTG
```

FIG. 7X

scrFI
mvaI
ecoRII
ecoRII
dsaV
bstNI
bssKI
apyI
haeIII/palI
mscI/balI
haeI
eaeI
hphI
cfrI
bsmAI
bsaI
hphI
ddeI
dpnI
sau3AI
mboI/ndeII
dpnII
haeI
bstXI
styI haeIII/palI
bsaJI
mnlI
tsp509I
tspRI
mspI
cfr10I/bsrFI
bsaWI
styI sau96I hpaII
ncoI haeIII/palI
dsaI asuI ageI
bsaJI pflMI
bspMI nlaIII bslI
bsmFI
taiI
hinlI/acyI
ahaII/bsaHI
aatII
mnlI maeII
tru9I
mseI
ahaIII/draI
bslI
hgiAI/aspHI
sau3AI
mboI/ndeII tru9I
dpnII bsp1286
dpnI bsiHKAI tsp509I
pvuI/bspCI mseI nlaIII
mcrI bmyI aseI/asnI/vspI
bsiEI apaLI/snoI rcaI apoI
tsp509I alw44I/snoI bspHI tsp509I
sau96I
avaII
asuI
mwoI
cac8I
tspRI mnlI nlaIII nlaIV ddeI sfaNI
GGGGTTTCAC CATATTGGCC AGGCTGGTCT CCAACTCCTA ATCTCAGGTG ATCTACCCAC CTTGGCCTCC CAAATTGCTG GGATTACAGG CGTGAACCAC
CCCCAAAGTG GTATAACCGG TCCGACCAGA GGTTGAGGAT TAGAGTCCAC TAGATGGGTG GAACCGGAGG GTTTAACGAC CCTAATGTCC GCACTTGGTG
TGCTCCCTTC CCTGTCCTTC TGATTTTAAA ATAACTATAC CAGCAGGAGG ACGTCCAGAC ACAGCATAGG CTACCTGCCA TGGCCCAACC GGTGGGACAT
ACGAGGGAAG GGACAGGAAG ACTAAAATTT TATTGATATG GTCGTCCTCC TGCAGGTCTG TGTCGTATCC GATGGACGGT ACCGGGTTGG CCACCCTGTA
301
401
501
^start of linker 2
^end of linker 2

FIG. 7Y

```
                                                                                                   scrFI
                                                                                                   mvaI
                                                                                                   ecoRII
                                                                                                   dsaV nlaIV
                                                                                                   bstNI
                                                                                                   bssKI
                                                                                                   bsaJI
                                                  bsrBI                               mspI         apyI hgiCI
    nlaIII   aluI                     tsp509I     aciI       tsp509I                  hpaII       mwoI  banI
601 CATGGTCATA GCTGTTTCCT GTGTGAAATT GTTATCCGCT CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT AAAGCCTGGG GTGCCTAATG
    CTACCAGTAT CGACAAAGGA CACACTTTAA CAATAGGCGA GTGTTAAGGT GTGTTGTATG CTCGGCCTTC GTATTTCACA TTTCGGACCC CACGGATTAC

                                                                                                            aciI
                                                                                                           thaI
                                                                                                           fnuDII/mvnI
                                                                                                           bstUI
                                                                                                           bsh1236I
                                                                                                          hinPI
                                                                                                          hhaI/cfoI
                                                                                                   eaeI    thaI
                  tsp509I   mwoI                                               aluI     tru9I      cfrI    fnuDII/mvnI
                 tru9I      hinPI                                             pvuII     mseI   tfiI haeIII/palI
      maeIII     mseI       hhaI/cfoI cac8I                                   mspA1I/nspBII   hinfI  bslI bstUI
   mnlI         aseI/asnI/vspI     tspRI aciI    bsrI                       cac8I      aseI/asnI/vspI      bsh1236I
701 AGTGAGGTAA CTCACATTAA TTGCGTTGCG CTCACTGCCC GCTTTCCAGT CGGGAAACCT GTCGTGCCAG CTGGATTAAT GAATCGGCCA ACGCGCGGGG
    TCACTCCATT GAGTGTAATT AACGCAACGC GAGTGACGGG CGAAAGGTCA GCCCTTTGGA CAGCACGGTC GACCTAATTA CTTAGCCGGT TGCGCGCCCC

                                                                                          mwoI
                         earI/ksp632I                          jinPI                fnu4HI/bsoFI
                        sapI                                   hhaI/cfoI             aciI   aciI
                      hinPI   aciI                  pleI  tseI     mcrI          tseI     bsrBI
     aciI             hhaI/cfoI                     hinfI fnu4HI/bsoFI           fnu4HI/bsoFI
   mnlI       mwoI   haeII mboII      mnlI      tspRI     bbvI    bsiEI          bbvI   cac8I        aluI          aciI
801 AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG GTCGTTCGGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG
    TCTCCGCCAA ACGCATAACC CGCGAGAAGG CGAAGGAGCG AGTGACTGAG CGACGCGAGC CAGCAAGCCG ACGCCGCTCG CCATAGTCGA GTGAGTTTCC
```

FIG. 7Z

```
                                                                                    scrFI
                                                                                    mvaI                 mwoI
                                                                                    ecoRII               thaI
                                                                                    dsaV                 fnuDII/mvnI
                                                                                    bstNI                bstUI
                                                      nlaIII            bslI        bssKI                bsh1236I
                                                     nspHI            cac8I         apyI   bslI         aciI   cac8I
                          tfiI                       nspI            haeIII/palI  HaeIII/palI         fnu4HI/bsoFI
                          hinfI                      aflIII         haeI        haeI   nlaIV         haeIII/palI
901 CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT
    GCCATTATGC CAATAGGTGT CTTAGTCCCC TATTGCGTCC TTTCTTGTAC ACTCGTTTTC CGGTCGTTTT CCGGTCCTTG GCATTTTTCC GGCGCAACGA

                                                                                                           scrFI
                                                                                                           mvaI
                                                                                                           ecoRII
                                                                                                           dsaV
                                                       hgaI                                                bstNI
                       aciI                             drdI                                               bssKI
                   nlaIV                sfaNI         taqI    smlI      mnlI                               apyI
001 GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
    CCGCAAAAAG GTATCCGAGG CGGGGGGACT GCTCGTAGTG TTTTTAGCTG CGAGTTCAGT CTCCACCGCT TTGGGCTGTC CTGATATTTC TATGGTCCGC

          scrFI
          mvaI
          ecoRII
          dsaV
          bstNI                                                 bciVI
          bssKI                                               mspI
          apy           bssSI hinPI                    bslI   hpaII                                     hinPI
         bsaJI  aluI mnlI     hhaI/cfoI                aciI  bsaWI                                      hhaI/cfoI
                                                      fnu4HI/bsoFI         aciI                       haeII
101 TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
    AAAGGGGGAC CTTCGAGGGA GCACGCGAGA GGACAAGGCT GGGACGGCGA ATGGCCTATG GACAGGCGGA AAGAGGGAAG CCCTTCGCAC CGCGAAAGAG

                                                                     hgiAI/aspHI                    tseI
                                                                     bsp1286                         fnu4HI/bsoFI
                                                                     bsiHKAI                      mspAlI/nspBII   mspI
                                                                     bmyI                         aciI  hinPI     hpaII
                                                                     apaLI/snoI                mcrI bbvI         bsaWI
               scfI       ddeI                            aluI       alw44I/snoI               bsiEI    hhaI/cfoI
201 AATGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
    TTACGAGTGC GACATCCATA GAGTCAAGCC ACATCCAGCA AGCGAGGTTC GACCCGACAC ACGTGCTTGG GGGGCAAGTC GGGCTGGCGA CGCGGAATAG
```

FIG. 7AA

```
                                                              alwNI
                       mspI                                   alw26I/bsmAI
                       hpaII                                 tseI
                      scrFI                                  fnu4HI/bsoFI
                      nciI                                   bbvI
            pleI      dsaV                                tseI      bsrI
            hinfI     cauII                          bsrI fnu4HI/bsoFI  maeIII
  maeIII       smlI       bssKi                     tspRI bbvI      tspRI                    mnlI        aciI
301 CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
    GCCATTGATA GCAGAACTCA GGTTGGGCCA TTCTGTGCTG AATAGCGGTG ACCGTCGTCG GTGACCATTG TCCTAATCGT CTCGCTCCAT ACATCCGCCA

                                                                                                        sau3AI
          mspI                                                                    hinPI       sau3AI    mboI/nde
          hpaII                                                                  thaI        mboI/ndeII dpnII
        sau3AI                                                        tseI       fnuDII/mvnI dpnII      dpnI
        mboI/ndeII                                                    fnu4HI/bsoFI hhaI/cfoI dpnI       alwI
        dpnII                                                         bbvI      bstUI       bstYI/xhoII bstYI/xhc
        dpnI               mspAlI/nspBII                                                     alwI smlI mboII
   aluI   alwI             aciI     aciI                      cac8I   mwoI      bsh1236I
501 TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA
    AACCATCGAG AACTAGGCCG TTTGTTTGGT GGCGACCATC GCCACCAAAA AAACAAACGT TCGTCGTCTA ATGCGCGTCT TTTTTTCCTA GAGTTCTTCT

                                                                                                      sau3AI
                                                                                                      mboI/ndeII
                                                                                         mboII        dpnII
                                                                                        sau3AI        dpnI
                                                                                        mboI/ndeII    alwI
     sau3AI                                                   tru9I                     dpnII     rmaI
     mboI/ndeII                                               mseI          nlaIII      dpnI      maeI
     dpnII                            ddeI                 taiI            rcaI        bstYI/xhoII bstYI/xhoII
     dpnI                       hgaI    tspRI              maeII           bspHI       alwI  hphI bfaI
601 TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC
    AGGAAACTAG AAAAGATGCC CCAGACTGCG AGTCACCTTG CTTTTGAGTG CAATTCCCTA AAACCAGTAC TCTAATAGTT TTTCCTAGAA GTGGATCTAG
```

FIG. 7BB

```
          tru9I
        tsp509I
     tru9I                      tru9I                                                                  nlaIV
     mseI mseI                  mseI                                                         tru9I     hgiCI
   ahaIII/draI                ahaIII/draI                                           maeIII   mseI   tspRI banI    ddeI
701 CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG
    GAAAATTTAA TTTTTACTTC AAAATTTAGT TAGATTTCAT ATATACTCAT TTGAACCAGA CTGTCAATGG TTACGAATTA GTCACTCCGT GGATAGAGTC

                                                                                                bsrI tseI
   sau3AI                                                                                    sau96I  fnu4HI/bsoFI
   mboI/ndeII                             pleI                                               nlaIV    bbvI
   dpnII              fokI                hinfI                                              haeIII/palI
   dpnI               bstF5I              ahdI/eam1105I                          mnlI        asuI tspRI  bsrDI
801 CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT GGCCCCAGTG CTGCAATGAT
    GCTAGACAGA TAAAGCAAGT AGGTATCAAC GGACTGAGGG GCAGCACATC TATTGATGCT ATGCCCTCCC GAATGGTAGA CCGGGGTCAC GACGTTACTA

      bsmAI
      bsai
    thaI
    fnuDII/mvnI        mspI bpmI/gsuI                        mspI
    bstUI              hpaII                               mwoI    haeIII/palI     sau96I                fokI
    bsh1236I          cfr10I/bsrFI                         bglI  sau96I  hinPI     avaII              mnlI
   aciI             hphI  nlaIV                       cac8I hpaII asuI   hhaI/cfoI  asuI           aciI  bstF5I
901 ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC CTGCAACTTT ATCCGCCTCC
    TGGCGCTCTG GGTGCGAGTG GCCGAGGTCT AAATAGTCGT TATTTGGTCG GTCGGCCTTC CCGGCTCGCG TCTTCACCAG GACGTTGAAA TAGGCGGAGG

                      scrFI
                      nciI
                      mspI                                           taiI
                      hpaII                                        psp1406I
              tsp509I dsaV     rmaI                             hinPI maeII        tseI     mslI
            tru9I     cauII    maeI                tru9I        hhaI/cfoI          fnu4HI/bsoFI
            mseI      bssKI    bfaI                mseI       mstI  aclI       bsrDI   cac8I        tsp45I
    bsrI  aseI/asnI/vspI     aluI              bsrI           aviII/fspI      mwoI  bbvI    sfaNI   maeIII
001 ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG CGCAACGTTG TTGCCATTGC TGCTGGCATC GTGGTGTCAC
    TAGGTCAGAT AATTAACAAC GGCCCTTCGA TCTCATTCAT CAAGCGGTCA ATTATCAAAC GCGTTGCAAC AACGGTAACG ACGACCGTAG CACCACAGTG
```

FIG. 7CC

```
                                                                  sau3AI
                                                                  mboI/ndeII
                         nlaIV        sau3AI                      dpnII
                       mspI           mboI/ndeII                  dpnI                                      sau96I
                     bsaWI            dpnII                   nlaIII                                        avaII
                  aluI hpaII          dpnI              maeIII    nlaIII                  aciI  aluI        asuI
101 GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTGCAAA AAAGCGGTTA GCTCCTTCGG
    CGAGCAGCAA ACCATACCGA AGTAAGTCGA GGCCAAGGGT TGCTAGTTCC GCTCAATGTA CTAGGGGGTA CAACACGTTT TTTCGCCAAT CGAGGAAGCC

      sau3AI
      mboI/ndeII
      dpnII                aciI
     pvuI/bspCI           fnu4HI/bsoFI                         tspRI
     mcrI                haeIII/palI                       tseI                                 fokI
     bsiEI              eaeI                   nlaIII      fnu4HI/bsoFI                         bstF5I
   mnlI dpnI            cfrI   tspRI           mslI        bbvI         tsp509I            nlaIII          sfaNI
201 TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC
    AGGAGGCTAG CAACAGTCTT CATTCAACCG GCGTCACAAT AGTGAGTACC AATACCGTCG TGACGTATTA AGAGAATGAC AGTACGGTAG GCATTCTACG

                                                                                  hgaI
                                                                                 hinlI/acyI
                                                                                 ahaII/bsaHI
                                                                               mspI                      hinPI
                                                                               hpaII                     hhaI/cfoI
                                                              mcrI            scrFI                     thaI
                                                              bsiEI           nciI                      fnuDII/mvnI
       bsrI      rsaI                                         bcgI            dsaV                      bstUI
     tsp45I     scaI                                        fnu4HI/bsoFI      cauII                     bsh1236I
     maeIII hphI csp6I                 ddeI                 aciI              bssKI                    aciI
301 TTTTCTGTGA CTGGTGAGTA CTCAACCAAG TCATTCTGAG AATAGTGTAT GCGGCGACCG AGTTGCTCTT GCCCGGCGTC ATCACGGGAT AATACCGCGC
    AAAAGACACT GACCACTCAT GAGTTGGTTC AGTAAGACTC TTATCACATA CGCCGCTGGC TCAACGAGAA CGGGCCGCAG TAGTGCCCTA TTATGGCGCG

                                                                                         sau3AI
                                           taiI                     sau3AI               mboI/ndeII
                         hgiAI/aspHI      maeII                     mboI/ndeII           dpnII
                         bsp1286         psp1406I                   dpnII                dpnI
                  tru9I  bsiHKAI         aclI                       dpnI     mspAlI/nspBII bsrI
                  mseI   bmyI          xmnI                       bstYI/xhoII            alwI    taqI
                ahaIII/draI            asp700      mboII       smlI alwI      aciI      bstYI/xhoII     maeIII
401 CACATAGCAG AACTTTAAAA GTGCTCATCA TTGGAAAACG TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG AGATCCAGTT CGATGTAACC
    GTGTATCGTC TTGAAATTTT CACGAGTAGT AACCTTTTGC AAGAAGCCCC GCTTTTGAGA GTTCCTAGAA TGGCGACAAC TCTAGGTCAA GCTACATTGG
```

FIG. 7DD

```
        hgiAI/aspHI
        bsp1286          eco57I
        bsiHKAI        mboII
        bmyI          sau3AI
        apaLI/sncI    mboI/ndeII
        alw44IsncI    dpnII                                                       aciI
     bssSI            dpnI      sfaNI        hphI         hphI                 fnu4HI/bsoFI
501 CACTCGTGCA CCCAACTGAT CTTCAGCATC TTTTACTTTC ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG CCGCAAAAAA GGGAATAAGG
    GTGAGCACGT GGGTTGACTA GAAGTCGTAG AAAATGAAAG TGGTCGCAAA GACCCACTCG TTTTTGTCCT TCCGTTTTAC GGCGTTTTTT CCCTTATTCC

                                                                                 nlaIII
                                                                                rcaI     bciVI
                                 mboII                                          bspHI aciI
      mslI                     earI/ksp632I  sspI                             bsmAI  bsrBI
601 GCGACACGGA AATGTTGAAT ACTCATACTC TTCCTTTTTC AATATTATTG AAGCATTTAT CAGGGTTATT GTCTCATGAG CGGATACATA TTTGAATGTA
    CGCTGTGCCT TTACAACTTA TGAGTATGAG AAGGAAAAAG TTATAATAAC TTCGTAAATA GTCCCAATAA CAGAGTACTC GCCTATGTAT AAACTTACAT

                            hinPI
                           thaI
                           fnuDII/mvnI
                           bstUI                          taiI
                           bsh1236I                       maeII
                          aciI                          hinlI/acyI                nlaIII
                      nnaIV hhaI/cfoI                   ahaII/bsaHI             rcaI      tru9I
                                                        aatII ddeI              bspHI     mseI
701 TTTAGAAAAA TAAACAAATA GGGGTTCCGC GCACATTTCC CCGAAAAGTG CCACCTGACG TCTAAGAAAC CATTATTATC ATGACATTAA CCTATAAAA
    AAATCTTTT ATTTGTTTAT CCCCAAGGCG CGTGTAAAGG GGCTTTTCAC GGTGGACTGC AGATTCTTTG GTAATAATAG TACTGTAATT GGATATTTT

                                            thaI                                       mspI
                                            fnuDII/mvnI                               scrFI
                                            bsh1236I                                  nciI esp3I
                                           hinPI                                      dsaV bsmBI
            sau96I    mboII                thaI                                   tseI  cauII
            haeIII/palI                    fnuDII/mvnI                            fnu4HI/bsoFI
           eco0109I/draII                  bstUI                               nlaIII    bssKI
          mnlI       bpuAI                 bsh1236I                            nsphI  aluI hpaII    tsp45I
       bssSI asuI    bbsI             mnlI hhaI/cfoI hphI     hphI    mnlI     nspI bbvI bslI bsmAI maeIII
801 TAGGCGTATC ACGAGGCCCT TTCGTCTTCA AGAATACTGC CTCGCGCGTT TCGGTGATGA CGGTGAAAAC CTCTGACACA TGCAGCTCCC GGAGACGGTC
    ATCCGCATAG TGCTCCGGGA AAGCAGAAGT TCTTATGACG GAGCGCGCAA AGCCACTACT GCCACTTTTG GAGACTGTGT ACGTCGAGGG CCTCTGCCAG
```

FIG. 7EE

```
                                 scrFI
                                 ncil
                                 mspI                          hgaI
                                 hpaII                        thaI
                                 dsaV                         fnuDII/mvnI
                             sfaNI                            bstUI   aciI
                             fokI  cauII                      bsh1236I
                             bstF5I                           hinPI  mspAlI/nspBII
        aluI             aciI    bssKI     drdI               hhaI/cfoI
901 ACAGCTTGTC TGTAAGCGGA TGCCGGGAGC AGACAAGCCC GTCAGGGCGC GTCAGCGGGT GTTGGCGGGT GTCGGGGCGC AGCCATGACC CAGTCACGTA
    TGTCGAACAG ACATTCGCCT ACGGCCCTCG TCTGTTCGGG CAGTCCCGCG CAGTCGCCCA CAACCGCCCA CAGCCCCGCG TCGGTACTGG GTCAGTGCAT

                                                                 mwoI
                                                                 bstAPI
                                                               hgiAI/aspHI
                                                               bsp1286
                                                               bsiHKAI
                                    sfaNI              ddeI    bmyI   ndeI
         mwoI        tru9I     fnu4HI/bsoFI          rsaI      apaLI/snoI
         aciI        mseI      aciI                  csp6I     alw44I/snoI aciI         aciI     sfaNI
001 GCGATAGCGG AGTTGGCTTA ACTATGCGGC ATCAGAGCAG ATTGTACTGA GAGTGCACCA TATGCGGTGT GAAATACCGC ACAGATGCGT AAGGAGAAAA
    CGCTATCGCC TCAACCGAAT TGATACGCCG TAGTCTCGTC TAACATGACT CTCACGTGGT ATACGCCACA CTTTATGGCG TGTCTACGCA TTCCTCTTTT

        mwoI
        hinPI
        hhaI/cfoI
       nlaIV
       narI                                                    sau3AI
       kasI                                                    mboI/ndeII
       hinlI/acyI                                              dpnII
       hgiCI                                                   dpnI      haeIII/palI          cac8I
       haeII                                                  pvuI/bspCI sau96I              aluI
       sfaNI   eheI       mwoI                                mcrI      asuI mboII          pvuII
       mwoI    banI       bglI                                bsiEI   cac8I  earI/ksp632I    mspAlI/nspBII   fokI
     aciI      ahaII/bsaHI                                   mwoI     aciI  mnlI          cac8I            bstF5I
101 TACCGCATCA GGCGCCATTC GCCATTCAGG CTACGCAACT GTTGGGAAGG GCGATCGGTG CGGGCCTTCT CGCTATTACG CCAGCTGGCG AAGGGGGGT
    ATGGCGTAGT CCGCGGTAAG CGGTAAGTCC GATGCGTTGA CAACCCTTCC CGCTAGCCAC GCCCGGAGAA GCGATAATGC GGTCGACCGC TTCCCCCCTA
```

FIG. 7FF

```
                                     scrFI
                                     mvaI
                                     ecoRII
                                     dsaV
                                     bstNI                                       tspRI
                                     bssKI                  taiI              bsrI
       tseI                          bsaJI            tsp45I                haeIII/palI
       fnu4HI/bsoFI tru9I            apyI             maeIII              eaeI
       bbvI         mseI      maeIII               bsrI     maeII         cfrI
   201 GTGCTGCAAG GCGATTAAGT TGGGTAACGC CAGGGTTTTC CCAGTCACGA CGTTGTAAAA CGACGGCCAG TGCC
       CACGACGTTC CGCTAATTCA ACCCATTGCT GTCCCAAAAG GGTCAGTGCT GCAACATTTT GCTGCCGGTC ACGG
```

FIG. 7GG

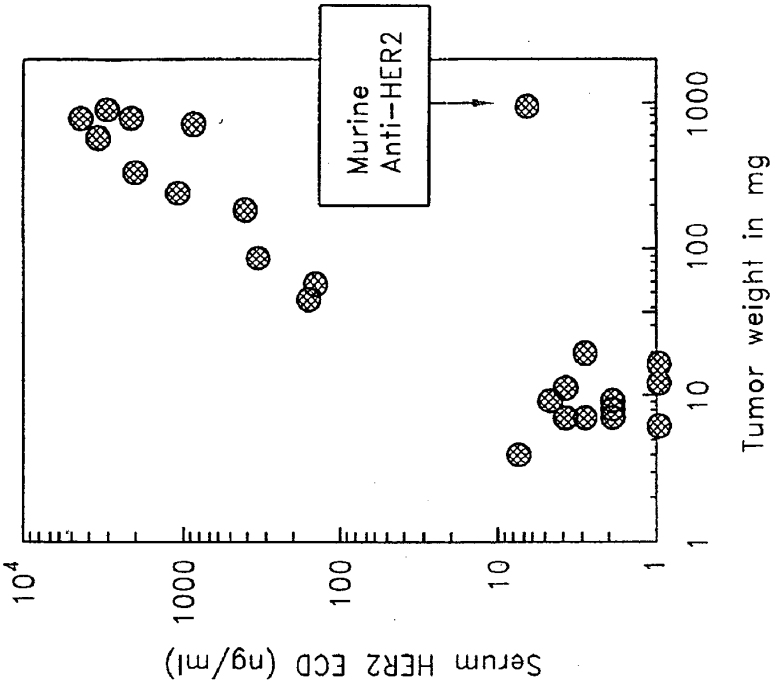
Murine
Anti-HER2
Serum HER2 ECD (ng/ml)
10^4
1000
100
10
1
Tumor weight in mg
1000
100
10
1

FIG. 8B

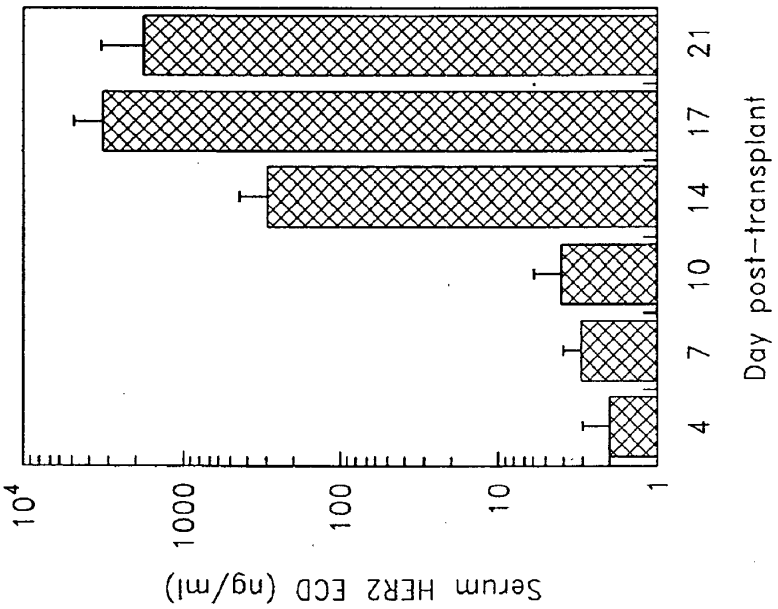
Serum HER2 ECD (ng/ml)
10^4
1000
100
10
1
Day post-transplant
4
7
10
14
17
21

FIG. 8A

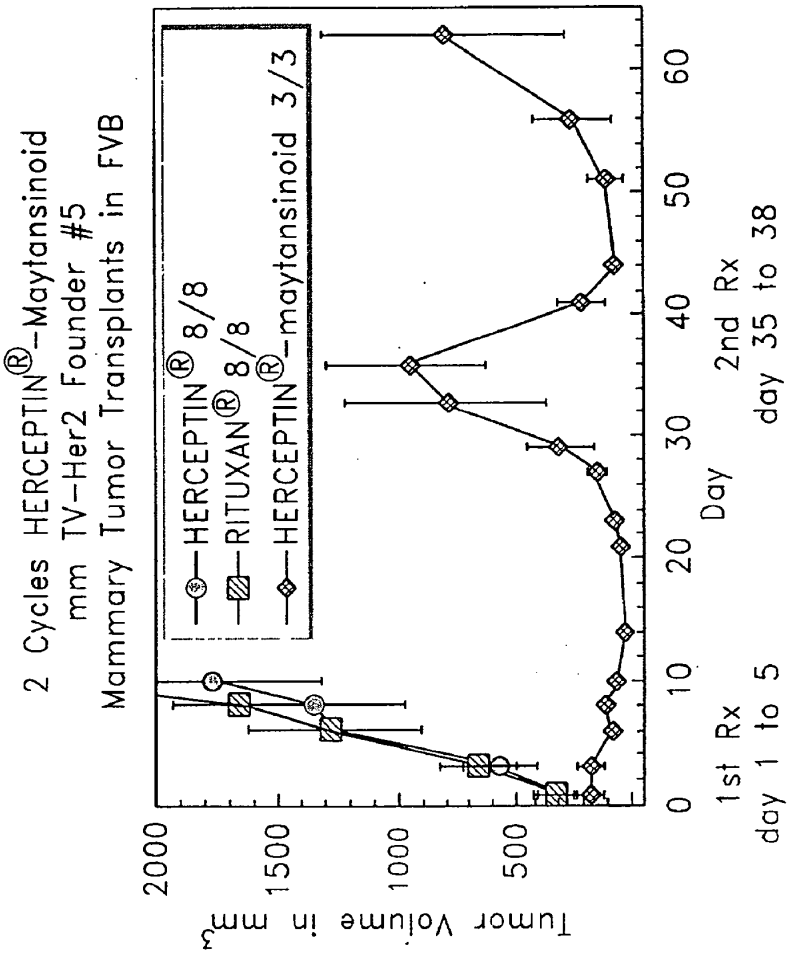
2 Cycles HERCEPTIN®-Maytansinoid
mm TV-Her2 Founder #5
Mammary Tumor Transplants in FVB
HERCEPTIN® 8/8
RITUXAN® 8/8
HERCEPTIN®-maytansinoid 3/3
Tumor Volume in mm3
2000
1500
1000
500
0
10
20
30
40
50
60
Day
1st Rx
day 1 to 5
2nd Rx
day 35 to 38

FIG. 9

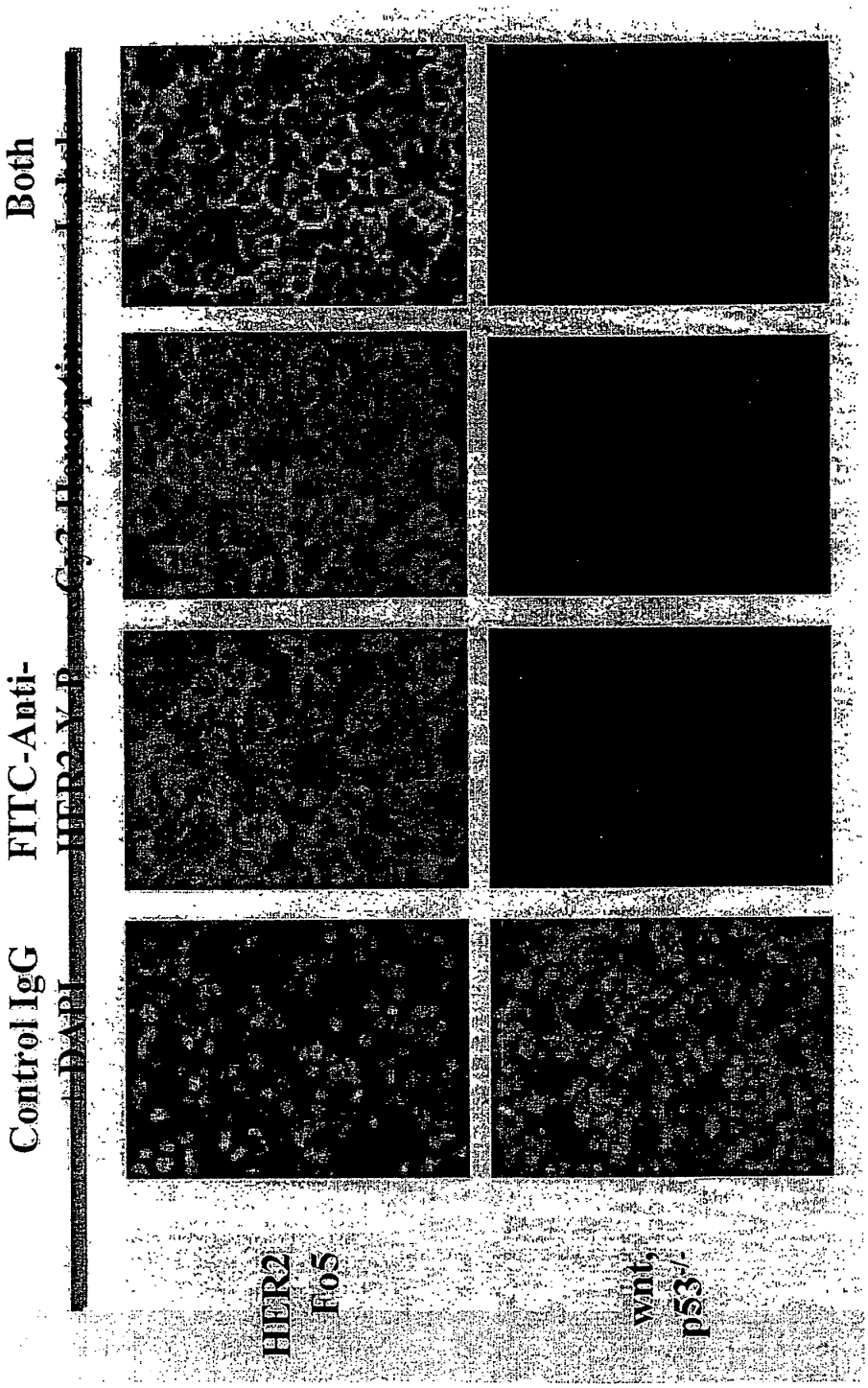

FIG. 10

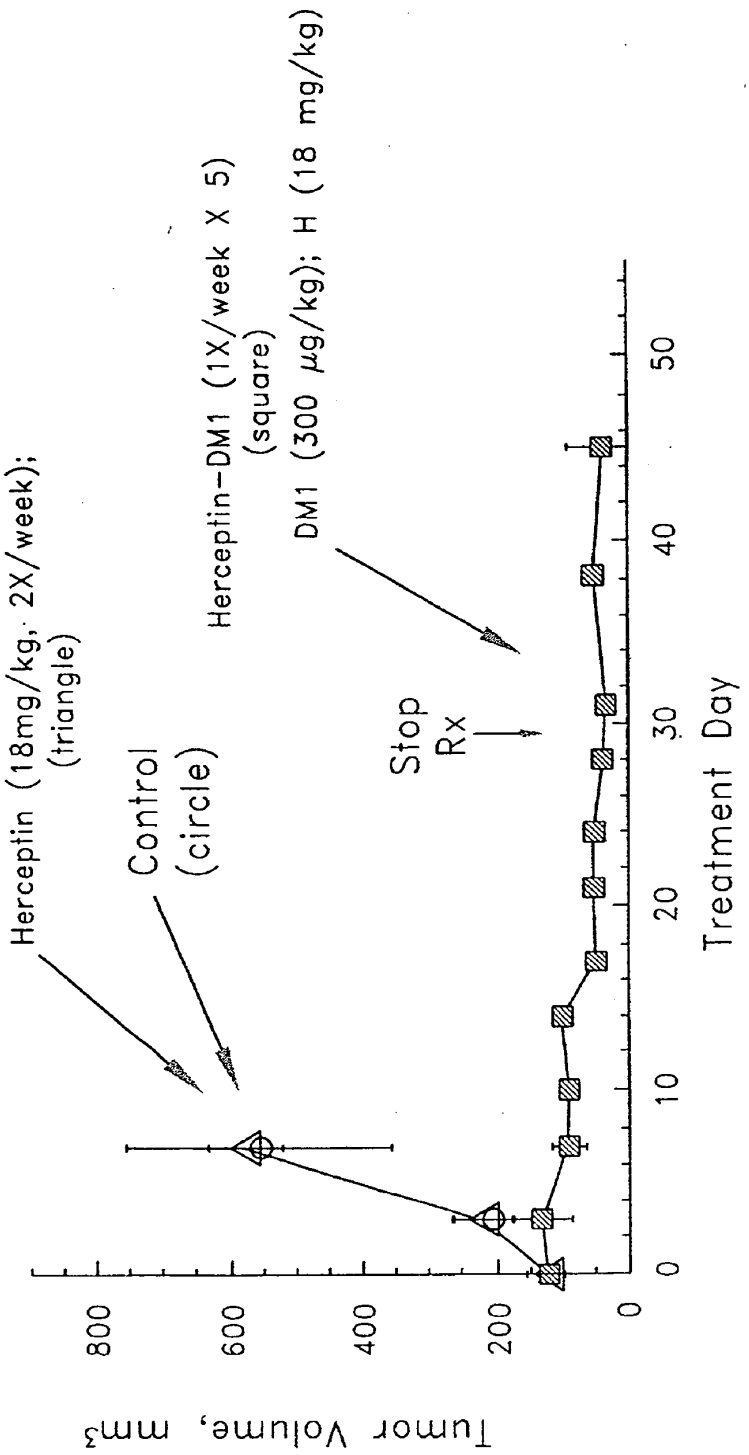
Herceptin (18mg/kg, 2X/week); (triangle)
Control (circle)
Herceptin–DM1 (1X/week X 5) (square)
DM1 (300 μg/kg); H (18 mg/kg)
Stop Rx
Tumor Volume, mm3
0
200
400
600
800
0
10
20
30
40
50
Treatment Day

FIG. 11

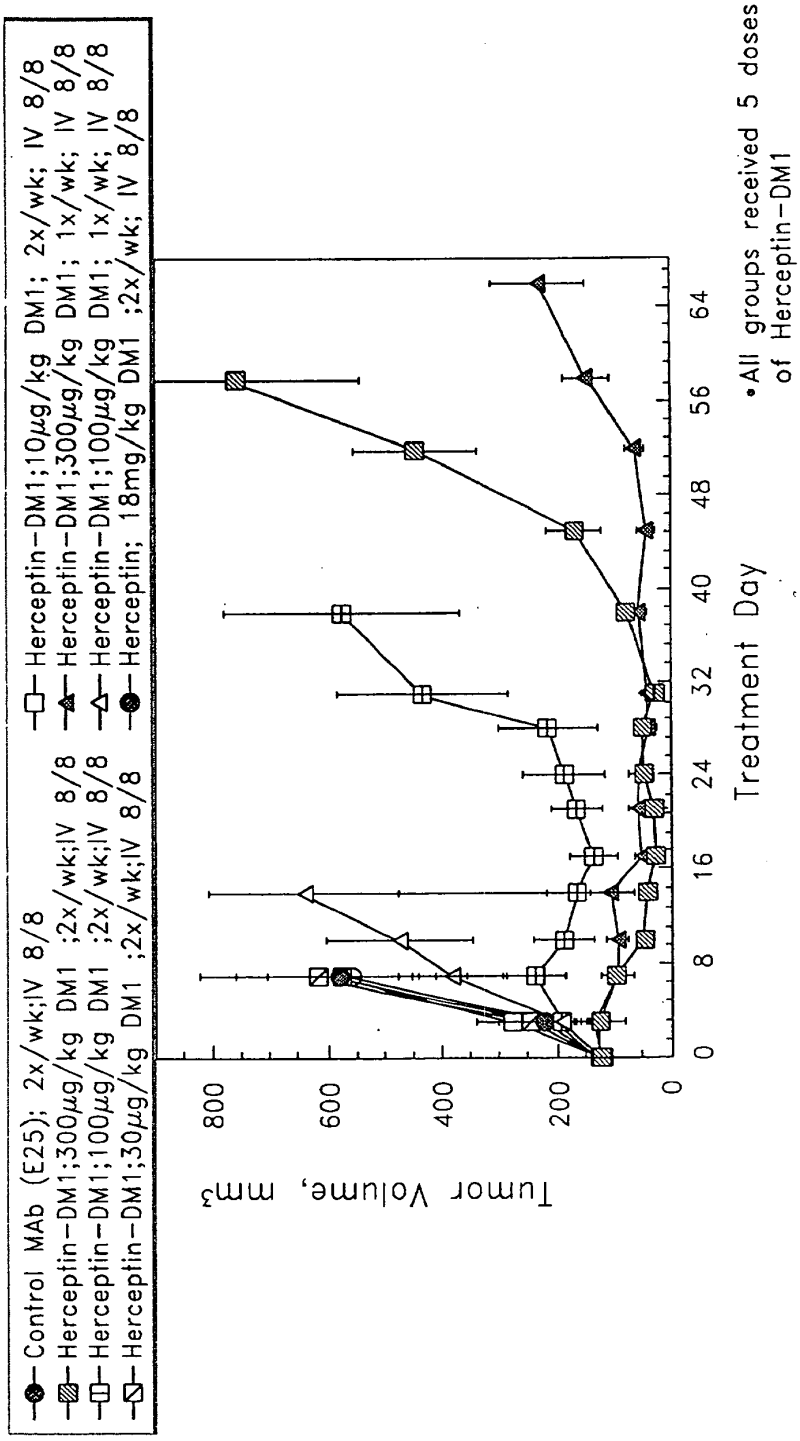
Control MAb (E25); 2x/wk;IV 8/8
Herceptin–DM1;300μg/kg DM1 ;2x/wk;IV 8/8
Herceptin–DM1;100μg/kg DM1 ;2x/wk;IV 8/8
Herceptin–DM1;30μg/kg DM1 ;2x/wk;IV 8/8
Herceptin–DM1;10μg/kg DM1; 2x/wk; IV 8/8
Herceptin–DM1;300μg/kg DM1; 1x/wk; IV 8/8
Herceptin–DM1;100μg/kg DM1; 1x/wk; IV 8/8
Herceptin; 18mg/kg DM1 ;2x/wk; IV 8/8
Tumor Volume, mm3
0
200
400
600
800
0
8
16
24
32
40
48
56
64
Treatment Day
•All groups received 5 doses of Herceptin–DM1

FIG. 12

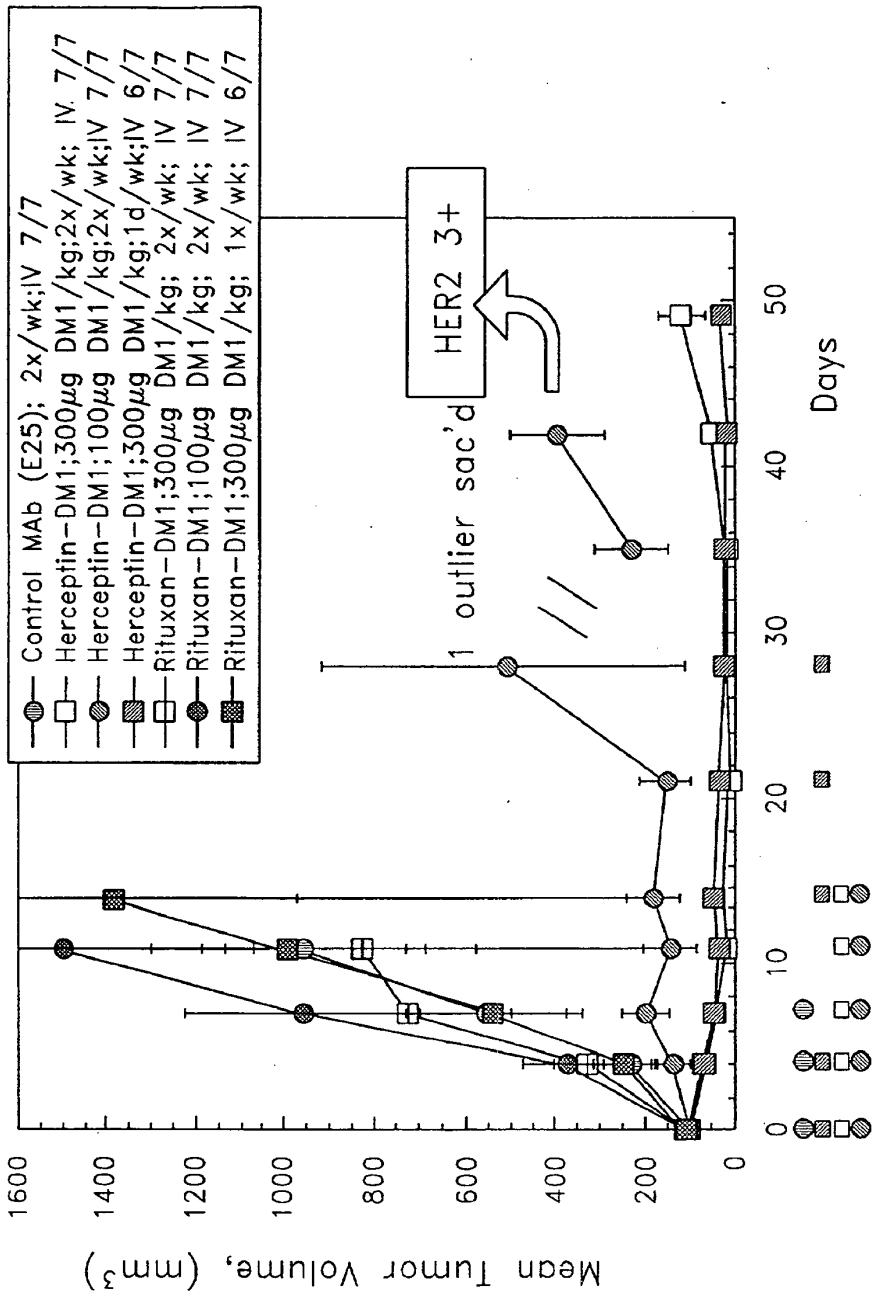
Control MAb (E25); 2x/wk;IV 7/7
Herceptin-DM1;300μg DM1/kg;2x/wk; IV. 7/7
Herceptin-DM1;100μg DM1/kg;2x/wk;IV 7/7
Herceptin-DM1;300μg DM1/kg;1d/wk;IV 6/7
Rituxan-DM1;300μg DM1/kg; 2x/wk; IV 7/7
Rituxan-DM1;100μg DM1/kg; 2x/wk; IV 7/7
Rituxan-DM1;300μg DM1/kg; 1x/wk; IV 6/7
HER2 3+
1 outlier sac'd
Mean Tumor Volume, (mm³)
Days
0
200
400
600
800
1000
1200
1400
1600
10
20
30
40
50

FIG. 13

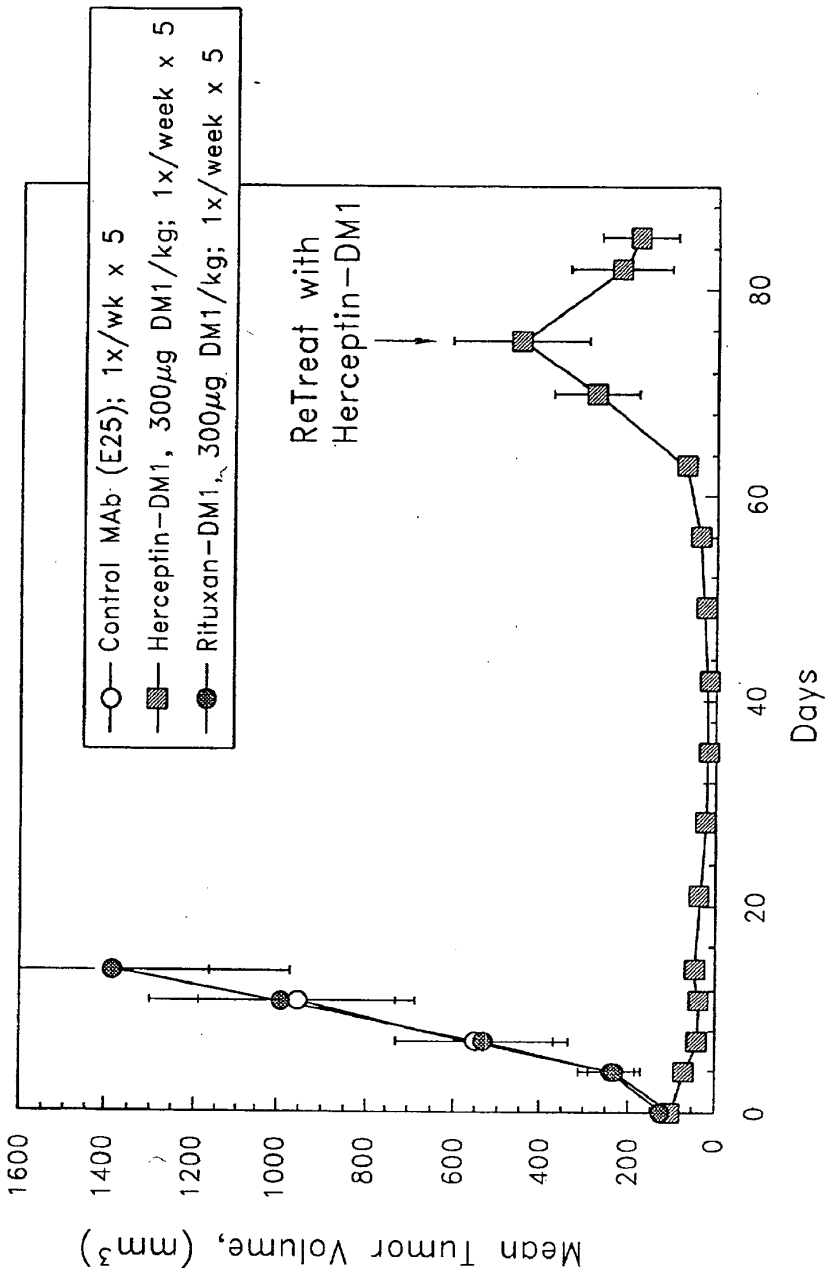
Control MAb (E25); 1x/wk x 5
Herceptin–DM1, 300μg DM1/kg; 1x/week x 5
Rituxan–DM1, 300μg DM1/kg; 1x/week x 5
ReTreat with Herceptin–DM1
Mean Tumor Volume, (mm³)
Days
0
200
400
600
800
1000
1200
1400
1600
0
20
40
60
80

FIG. 14

METHODS OF TREATMENT USING ANTI-ERBB ANTIBODY-MAYTANSINOID CONJUGATES

This application claims priority to U.S. Provisional Application Nos. 60/238,327, filed Oct. 5, 2000, 60/189,844 filed Mar. 16, 2000, and 60/327,563 filed Jun. 23, 2000 (converted from U.S. application Ser. No. 09/602,530) under 35 USC §119(e).

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention concerns methods of treatment, especially ErbB receptor-directed cancer therapies, using anti-ErbB receptor antibody-maytansinoid conjugates, and articles of manufacture suitable for use in such methods.

2. Description of the Related Art

1. Maytansine and Maytansinoids

Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinol and maytansinol analogues are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533, the disclosures of which are hereby expressly incorporated by reference.

Maytansine and maytansinoids are highly cytotoxic but their clinical use in cancer therapy has been greatly limited by their severe systemic side-effects primarily attributed to their poor selectivity for tumors. Clinical trials with maytansine had been discontinued due to serious adverse effects on the central nervous system and gastrointestinal system (Issel et al., *Can. Trtmnt. Rev.* 5:199–207 [1978]).

2. The ErbB Family of Receptor Tyrosine Kinases and Anti-ErbB Antibodies

Members of the ErbB family of receptor tyrosine kinases are important mediators of cell growth, differentiation and survival. The receptor family includes four distinct members, including epidermal growth factor receptor (EGFR or ErbB1), HER2 (ErbB2 or p185^{neu}), HER3 (ErbB3) and HER4 (ErbB4 or tyro2).

p185^{neu}, was originally identified as the product of the transforming gene from neuroblastomas of chemically treated rats. The activated form of the neu proto-oncogene results from a point mutation (valine to glutamic acid) in the transmembrane region of the encoded protein. Amplification of the human homologue of neu is observed in breast and ovarian cancers and correlates with a poor prognosis (Slamon et al., *Science,* 235:177–182 (1987); Slamon et al., *Science,* 244:707–712 (1989); and U.S. Pat No. 4,968,603). To date, no point mutation analogous to that in the neu proto-oncogene has been reported for human tumors. Overexpression of ErbB2 (frequently but not uniformly due to gene amplification) has also been observed in other carcinomas including carcinomas of the stomach, endometrium, salivary gland, lung, kidney, colon, thyroid, pancreas and bladder. See, among others, King et al., *Science,* 229:974 (1985); Yokota et al., *Lancet:* 1:765–767 (1986); Fukushigi et al., *Mol Cell Biol.,* 6:955–958 (1986); Geurin et al., *Oncogene Res.,* 3:21–31 (1988); Cohen et al., *Oncogene,* 4:81–88 (1989); Yonemura et al., *Cancer Res.,* 51:1034 (1991); Borst et al., *Gynecol. Oncol.,* 38:364 (1990); Weiner et al., *Cancer Res.,* 50:421–425 (1990); Kern et al., *Cancer Res.,* 50:5184 (1990); Park et al., *Cancer Res.,* 49:6605 (1989); Zhau et al., *Mol. Carcinog.,* 3:354–357 (1990); Aasland et al. *Br. J. Cancer* 57:358–363 (1988); Williams et al. *Pathobiology* 59:46–52 (1991); and McCann et al., *Cancer,* 65:88–92 (1990). ErbB2 may be overexpressed in prostate cancer (Gu et al. *Cancer Lett.* 99:185–9 (1996); Ross et al. *Hum. Pathol.* 28:827–33 (1997); Ross et al. *Cancer* 79:2162–70 (1997); and Sadasivan et al. *J. Urol.* 150:126–31 (1993)).

A spliced form of erbB2 oncogen encoding a constitutively tyrosine phosphorylated ErbB2 receptor is disclosed in PCT publication WO 00/20579, published on Apr. 13, 2000. The erbB2 protein encoded by the splice variant has an in frame deletion of 16 amino acids (CVDLDDKGC-PAEQRAS (SEQ ID NO: 11)), two of which are conserved cysteine residues.

Antibodies directed against the rat p185^{neu} and human ErbB2 protein products have been described. Drebin and colleagues have raised antibodies against the rat neu gene product, p185^{neu}. See, for example, Drebin et al., *Cell* 41:695–706 (1985); Myers et al., *Meth. Enzym.* 198:277–290 (1991); and WO94/22478. Drebin et al. *Oncogene* 2:273–277 (1988) report that mixtures of antibodies reactive with two distinct regions of p185^{neu} result in synergistic anti-tumor effects on neu-transformed NIH-3T3 cells implanted into nude mice. See also U.S. Pat. No. 5,824,311 issued Oct. 20, 1998.

Other anti-ErbB2 antibodies with various properties have been described in Tagliabue et al. *Int. J. Cancer* 47:933–937 (1991); McKenzie et al. *Oncogene* 4:543–548 (1989); Maier et al. *Cancer Res.* 51:5361–5369 (1991); Bacus et al. *Molecular Carcinogenesis* 3:350–362 (1990); Stancovski et al. *PNAS (USA)* 88:8691–8695 (1991); Bacus et al. *Cancer Research* 52:2580–2589 (1992); Xu et al. *Int. J. Cancer* 53:401–408 (1993); WO94/00136; Kasprzyk et al. *Cancer Research* 52:2771–2776 (1992);Hancock et al. *Cancer Res.* 51:4575–4580 (1991); Shawver et al. *Cancer Res.* 54:1367–1373 (1994); Arteaga et al. *Cancer Res.* 54:3758–3765 (1994); Harwerth et al. *J. Biol. Chem.* 267: 15160–15167 (1992); U.S. Pat. No. 5,783,186; and Klapper et al. *Oncogene* 14:2099–2109 (1997).

Hudziak et al., *Mol. Cell. Biol.* 9(3): 1165–1172 (1989) describe the generation of a panel of anti-ErbB2 antibodies which were characterized using the human breast tumor cell line SK-BR-3. Relative cell proliferation of the SK-BR-3 cells following exposure to the antibodies was determined by crystal violet staining of the monolayers after 72 hours. Using this assay, maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel reduced cellular proliferation to a lesser extent in this assay. The antibody 4D5 was further found to sensitize ErbB2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also U.S. Pat. No. 5,677,171 issued Oct. 14, 1997. The anti-ErbB2 antibodies discussed in Hudziak et al. are further characterized in Fendly et al. *Cancer Research* 50:1550–1558 (1990); Kotts et al. *In Vitro* 26(3):59A (1990); Sarup et al. *Growth Regulation* 1:72–82 (1991); Shepard et al. *J. Clin. Immunol.* 11(3):117–127 (1991); Kumar et al. *Mol. Cell. Biol.* 11(2):979–986 (1991); Lewis et al. *Cancer Immunol. Immunother.* 37:255–263 (1993); Pietras et al. *Oncogene* 9:1829–1838 (1994); Vitetta et al. *Cancer Research* 54:5301–5309 (1994); Sliwkowski et al. *J. Biol. Chem.* 269(20):14661–14665 (1994); Scott et al. *J. Biol. Chem.* 266:14300–5 (1991); D'souza et al. *Proc. Natl.*

Acad. Sci. 91:7202–7206 (1994); Lewis et al. *Cancer Research* 56:1457–1465 (1996); and Schaefer et al. *Oncogene* 15:1385–1394 (1997).

The murine monoclonal anti-HER2 antibody inhibits the growth of breast cancer cell lines that overexpress HER2 at the 2+ and 3+ level, but has no activity on cells that express lower levels of HER2 (Lewis et al., *Cancer Immunol. Immunother.* [1993]). Based on this observation, antibody 4D5 was humanized (Carter et al., *Proc. Natl. Acad. Sci.* USA 89: 4285–4289 [1992]). The humanized version designated HERCEPTIN® (huMAb4D5-8, rhuMAb HER2, U.S. Pat. No. 5,821,337) was tested in breast cancer patients whose tumors overexpress HER2 but who had progressed after conventional chemotherapy (Baselga et al., *J. Clin. Oncol.* 14:737–744 [1996]); Cobleigh et al., *J. Clin. Oncol.* 17: 2639–2648 [1999]). Most patients in this trial expressed HER2 at the 3+ level, though a fraction was 2+ tumors. Remarkably, HERCEPTIN® induced clinical responses in 15% of patients (complete responses in 4% of patients, and partial responses in 11%) and the median duration of those responses was 9.1 months. HERCEPTIN® received marketing approval from the Food and Drug Administration Sep. 25, 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the ErbB2 protein.

Homology screening has resulted in the identification of two other ErbB receptor family members; ErbB3 (U.S. Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus et al. *PNAS (USA)* 86:9193–9197 (1989)) and ErbB4 (EP Pat Appln No 599,274; Plowman et al., *Proc. Natl. Acad. Sci. USA,* 90:1746–1750 (1993); and Plowman et al., *Nature,* 366: 473–475 (1993)). Both of these receptors display increased expression on at least some breast cancer cell lines.

3. Maytansinoid-antibody Conjugates

In an attempt to improve their therapeutic index, maytansine and maytansinoids have been conjugated to antibodies specifically binding to tumor cell antigens. Immunoconjugates containing maytansinoids are disclosed, for example, in U.S. Pat. Nos. 5,208,020; 5,416,064 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. Liu et al., *Proc. Natl. Acad. Sci. USA* 93:8618–8623 (1996) described immunoconjugates comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an in vivo tumor growth assay. Chari et al. *Cancer Research* 52:127–131 (1992) describe immunoconjugates in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/neu oncogene. The cytotoxicity of the TA.1-maytansonoid conjugate was tested in vitro on the human breast cancer cell line SK-BR-3, which expresses 3×10^5 HER-2 surface antigens per cell. The drug conjugate achieved a degree of cytotoxicity similar to the free maytansinoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

Although HERCEPTIN® is a breakthrough in treating patients with ErbB2-overexpressing breast cancers that have received extensive prior anti-cancer therapy, generally approximately 85% of the patients in this population fail to respond, or respond only poorly, to HERCEPTIN® treatment, and in the clinical trial preceding marketing approval, the median time to disease progression in all treated patients was only 3.1 months. Therefore, there is a significant clinical need for developing farther HER2-directed cancer therapies for those patients with HER2-overexpressing tumors or other diseases associated with HER2 expression that do not respond, or respond poorly, to HERCEPTIN® treatment.

SUMMARY OF THE INVENTION

The present invention is based on the unexpected experimental finding that HERCEPTIN®-maytansinoid conjugates are highly effective in the treatment of HER2 (ErbB2) overexpressing tumors that do not respond, or respond poorly, to HERCEPTIN® therapy. The anti-ErbB2-maytansinoid conjugates of the present invention are expected to have superior clinical activity compared to treatment with HERCEPTIN® alone, including a better objective response rate and/or longer duration of response and/or increased survival.

In one aspect, the present invention concerns a method for the treatment of a tumor in a mammal, wherein the tumor is characterized by the overexpression of an ErbB receptor and does not respond or responds poorly to treatment with a monoclonal anti-ErbB antibody, comprising administering to the mammal a therapeutically effective amount of a conjugate of the anti-ErbB antibody with a maytansinoid.

In a preferred embodiment, the patient is human. In another preferred embodiment, the ErbB receptor is (human) ErbB2 (HER2). The method is not limited by the mechanism of action of the anti-ErbB antibody used. Thus, the anti-ErbB antibody may, for example, have growth inhibitory properties and/or may induce cell death and/or apoptosis. In a particularly preferred embodiment, the method concerns the treatment of cancer including, without limitation, breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer. Preferably the cancer is breast cancer, in particular, breast cancer which overexpresses ErbB2 at a 2+ level or above, more preferably at a 3+ level. A preferred group of antibodies has a biological characteristic of a 4D5 monoclonal antibody, or binds essentially the same epitope as a 4D5 monoclonal antibody, a humanized form of the murine monoclonal antibody 4D5 (ATCC CRL 10463) being particularly preferred.

The maytansinoid used in the conjugates of the present invention may be maytansine or, preferably, maytansinol or a maytansinol ester. The antibody and maytansinoid may be conjugated by a bispecific chemical linker, such as N-succinimidyl-4-(2-pyridylthio)propanoate (SPDP) or N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP). The linking group between the antibody and the maytansinoid may, for example, be a disulfide, thioether, acid labile, photolabile, peptidase labile, or esterase labile group.

In another aspect, the invention concerns an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an anti-ErbB antibody-maytansinoid conjugate, and further comprising a package insert or label indicating that the composition can be used to treat cancer characterized by overexpression of an ErbB receptor, preferably at a 2+ level or above.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the heavy chain variable region sequence of a humanized anti-HER2 antibody 2C4 (SEQ ID NO: 1) aligned with the heavy chain variable region sequences of antibody 574 (SEQ ID NO: 2) and the human subgroup V_HIII upon which the humanized sequences are based (SEQ ID NO: 3).

FIG. 2 shows the light chain variable region sequence of a humanized anti-HER2 antibody 2C4 (SEQ ID NO: 4) aligned with the light chain variable region sequences of antibody 574 (SEQ ID NO: 5) and the sequence of the human subgroup upon which the humanized sequences are based (SEQ ID NO: 6).

FIG. 3 shows the structure of the maytansinoid, designated "DM1." In the structure of DM1, "R" can be occupied by a variety of groups capable of forming a chemical bond with a selected linker. Preferably, "R" is an SH group or a protected derivative thereof, which forms an S—S bond with a linker, such as N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP).

FIG. 4 illustrates the structure of a HERCEPTIN®-DM1 conjugate.

FIG. 5 is the elution profile of HERCEPTIN®-DM1 conjugate on a Sephacryl S300 gel filtration column.

FIG. 6 shows the anti-proliferative effect of HERCEPTIN® and HERCEPTIN®-DM1 conjugate on SK-BR3 cells in vitro. As control, the unrelated monoclonal antibody RITUXAN® or RITUXAN®-DM1 conjugate was used.

FIG. 7A–GG shows the nucleotide sequence of a HER2 transgene plasmid construct (SEQ ID NO: 7) directing the expression of native human HER2 (ErbB2) in the mammary gland of a transgenic mouse. The reverse strand is also depicted (SEQ ID NO: 10). The figure includes the nucleotide sequence of HER2 (ErbB2) cDNA insert (SEQ ID NO: 8) as well as the deduced amino acid sequence of HER2 (ErbB2) (SEQ ID NO: 9), including the signal sequence. Within SEQ ID NO: 9, residues from about 22 to about 645, inclusive represent the HER2 (ErbB2) extracellular domain.

FIGS. 8A and B show that the amount of HER2 extracellular domain (ECD) shed into serum increases following transplant (FIG. 8A) and is proportional to the weight of the resulting tumor (FIG. 8B).

FIG. 9 illustrates the effect of HERCEPTIN®-DM1 on HER2-transgenic tumors. Two mm^3 pieces of MMTV-HER2-transgenic tumors were transplanted into the mammary fat pad of FVB mice. When tumors reached 250 mm^3, groups of 8 mice were injected i.v. on 5 consecutive days with a HERCEPTIN®-DM1 conjugate. Two other groups of mice were treated IP twice per week with 10 mg/kg of either HERCEPTIN® or RITUXAN®.

FIG. 10 shows that tumor cells originating from Founder 5 show binding to cy3-HERCEPTIN® and to an anti-tyrosine-phosphorylated-HER2 antibody. Antibodies were injected intravenously into transgenic mice and the next day tumors were collected and sectioned. Antibody binding was visualized by fluoresence microscopy.

FIG. 11 shows the effect of HERCEPTIN® and HERCEPTIN®-DM1 conjugate on the growth of HER2 transgenic tumor transplant. HERCEPTIN®-DM1 was administered once a week for 5 weeks at 300 μg DM1/kg or 18 mg/kg of HERCEPTIN®. HERCEPTIN® or a control mAb (RITUXAN®) was administered twice a week at 18 mg/kg.

FIG. 12 shows evaluation of doses and schedule of treatment with HERCEPTIN®-DM1 conjugate in HER2 transgenic tumor transplant in nude mice. HERCEPTIN®-DM1 conjugate was administered either twice a week at various doses (300, 100, 30 or 10 μg DM1/kg) or once a week at various doses (300 or 100 μg DM1/kg) for 5 weeks. HERCEPTIN® or a control mAb E25 (RITUXAN®) was administered twice a week at 18 mg/kg.

FIG. 13 shows the effect of different HERCEPTIN®-DM1 dosing regimens on HER2-transgenic tumors compared to matching doses of RITUXAN®-DM1. Mice with 100 mm^3 tumors were injected i.v. with HERCEPTIN®-DM1 or RITUXAN®-DM1 at doses of 100 or 300 μg DM1/kg twice a week or 300 μg DM1/kg once a week. All animals received 5 doses.

FIG. 14 is a comparison of the most effective observed dose of HERCEPTIN®-DM1 and RITUXAN®-DM1. A second dose of HERCEPTIN®-DM1 following the beginning of tumor regrowth was effective in shrinking the tumors again.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton et al., *Dictionary of Microbiology and Molecular Biology 2nd ed.*, J. Wiley & Sons (New York, N.Y. 1994). One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

An "ErbB receptor" or "ErbB" is a receptor protein tyrosine kinase which belongs to the ErbB receptor family and includes ErbB1 (EGFR), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4) receptors and other members of this family to be identified in the future. The definition specifically includes ErbB receptors encoded by spliced forms of the corresponding erbB oncogens, including, without limitation, the deletion variant of ErbB2 disclosed in PCT publication No. WO 00/20579 (published on Apr. 13, 2000). The ErbB receptor will generally comprise an extracellular domain, which may bind an ErbB ligand; a lipophilic transmembrane domain; a conserved intracellular tyrosine kinase domain; and a carboxyl-terminal signaling domain harboring several tyrosine residues which can be phosphorylated. The ErbB receptor may be a "native sequence" ErbB receptor or a functional derivative, such as an "amino acid sequence variant" thereof. Preferably the ErbB receptor is native sequence human ErbB receptor.

The terms "ErbB 1", "epidermal growth factor receptor" and "EGFR" are used interchangeably herein and refer to native sequence EGFR as disclosed, for example, in Carpenter et al. *Ann. Rev. Biochem.* 56:881–914 (1987), including naturally occurring mutant forms thereof (e.g. a deletion mutant EGFR as in Humphrey et al. *PNAS* (USA) 87:4207–4211 (1990)), and its functional derivatives, such as amino acid sequence variants. erbB1 refers to the gene encoding the EGFR protein product.

The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to native sequence human HER2 protein described, for example, in Semba et al., *PNAS (USA)* 82:6497–6501 (1985) and Yamamoto et al. *Nature* 319:230–234 (1986) (Genebank accession number X03363), and functional derivatives, such as amino acid sequence variants thereof. The term erbB2 refers to the gene encoding human HER2 and neu refers to the gene encoding rat $p185^{neu}$. Preferred HER2 is native sequence human HER2. Examples of antibodies which bind HER2 include MAbs 4D5 (ATCC CRL 10463), 2C4 (ATCC HB-12697), 7F3 (ATCC HB-12216), and 7C2 (ATCC HB 12215) (see, U.S. Pat. No. 5,772,997; WO98/77797; and U.S. Pat. No. 5,840,525, expressly incorporated herein by reference).

Humanized anti-HER2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®) as described in Table 3 of U.S. Pat. No. 5,821,337 expressly incorporated herein by reference; humanized 520C9 (WO93/21319). Human anti-HER2 antibodies are described in U.S. Pat. No. 5,772,997 issued Jun. 30, 1998 and WO 97/00271 published Jan. 3, 1997.

"ErbB3" and "HER3" refer to the receptor polypeptide as disclosed, for example, in U.S. Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus et al. *PNAS (USA)* 86:9193–9197 (1989), and functional derivatives, including amino acid sequence variants thereof. Examples of antibodies which bind HER3 are described in U.S. Pat. No. 5,968,511 (Akita and Sliwkowski), e.g. the 8B8 antibody (ATCC HB 12070) or a humanized variant thereof.

The terms "ErbB4" and "HER4" herein refer to the receptor polypeptide as disclosed, for example, in EP Pat Appln No 599,274; Plowman et al., *Proc. Natl. Acad. Sci. USA,* 90:1746–1750 (1993); and Plowman et al., *Nature,* 366:473–475 (1993), and functional derivatives, including amino acid sequence variants thereof such as the HER4 isoforms disclosed in WO 99/19488.

A "native" or "native sequence" EGFR, HER2, HER3 or HER4 polypeptide may be isolated from nature, produced by techniques of recombinant DNA technology, chemically synthesized, or produced by any combinations of these or similar methods. "Functional derivatives" include amino acid sequence variants, and covalent derivatives of the native polypeptides as long as they retain a qualitative biological activity of the corresponding native polypeptide. Amino acid sequence variants generally differ from a native sequence in the substitution, deletion and/or insertion of one or more amino acids anywhere within a native amino acid sequence. Deletional variants include fragments of the native polypeptides, and variants having N- and/or C-terminal truncations. Ordinarily, amino acid sequence variants will possess at least about 70% homology, preferably at least about 80%, more preferably at least about 90% homology with a native polypeptide.

"Homology" is defined as the percentage of residues in the amino acid sequence variant that are identical after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology. Methods and computer programs for the alignment are well known in the art. One such computer program is "Align 2", authored by Genentech, Inc., which was filed with user documentation in the United States Copyright Office, Washington, D.C. 20559, on Dec. 10, 1991.

By "ErbB ligand" is meant a polypeptide which binds to and/or activates an ErbB receptor. The ErbB ligand of particular interest herein is a native sequence human ErbB ligand such as Epidermal Growth Factor (EGF) (Savage et al., *J. Biol. Chem.* 247:7612–7621 (1972)); Transforming Growth Factor alpha (TGF-alpha) (Marquardt et al., *Science* 223:1079–1082 (1984)); amphiregulin also known as schwanoma or keratinocyte autocrine growth factor (Shoyab et al. *Science* 243:1074–1076 (1989); Kimura et al. *Nature* 348:257–260 (1990); and Cook et al. *Mol. Cell. Biol* 11:2547–2557 (1991)); betacellulin (Shing et al., *Science* 259:1604–1607 (1993); and Sasada et al. *Biochem. Biophys. Res. Commun.* 190:1173 (1993)); heparin-binding epidermal growth factor (HB-EGF) (Higashiyama et al., *Science* 251: 936–939 (1991)); epiregulin (Toyoda et al., *J. Biol. Chem.* 270:7495–7500 (1995); and Komurasaki et al. *Oncogene* 15:2841–2848 (1997)), a heregulin (see below); neuregulin-2 (NRG-2) (Carraway et al., *Nature* 387:512–516 (1997)); neuregulin-3 (NRG-3) (Zhang et al., *Proc. Natl. Acad. Sci.* 94:9562–9567 (1997)); or cripto (CR-1) (Kannan et al. *J. Biol. Chem.* 272(6):3330–3335 (1997)). ErbB ligands which bind EGFR include EGF, TGF-alpha, amphiregulin, betacellulin, HB-EGF and epiregulin. ErbB ligands which bind HER3 include heregulins. ErbB ligands capable of binding HER4 include betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3 and heregulins.

"Heregulin" (HRG) when used herein refers to a polypeptide which activates the ErbB2-ErbB3 and ErbB2-ErbB4 protein complexes (i.e. induces phosphorylation of tyrosine residues in the complex upon binding thereto). Various heregulin polypeptides encompassed by this term are disclosed in Holmes et al., *Science* 256:1205–1210 (1992); WO 92/20798; Wen et al.; *Mol. Cell. Biol.* 14(3):1909–1919 (1994) and Marchionni et al., *Nature* 362:312–318 (1993), for example. The term includes biologically active fragments and/or variants of a naturally occurring HRG polypeptide, such as an EGF-like domain fragment thereof (e.g. $HRG\beta_{177-244}$).

An "ErbB hetero-oligomer" herein is a noncovalently associated oligomer comprising at least two different ErbB receptors. Such complexes may form when a cell expressing two or more ErbB receptors is exposed to an ErbB ligand and can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski et al., *J. Biol. Chem.,* 269(20):14661–14665 (1994), for example. Examples of such ErbB hetero-oligomers include EGFR-HER2, HER2-HER3 and HER3-HER4 complexes. Moreover, the ErbB hetero-oligomer may comprise two or more HER2 receptors combined with a different ErbB receptor, such as HER3, HER4 or EGFR. Other proteins, such as a cytokine receptor subunit (e.g. gp130), may be included in the hetero-oligomer.

In the context of HER2 variants, such as HER2 fragments, the phrase "having the biological activity of a native human HER2" is used to refer to the qualitative ability of such fragments to induce tumor growth when overexpressed in an animal model (transgenic or non-transgenic) of the present invention.

"Tumor", as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

A cancer which "overexpresses" an ErbB receptor is one which has significantly higher levels of an ErbB receptor, such as HER2, at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. ErbB receptor overexpression

may be determined in a diagnostic or prognostic assay by evaluating increased levels of the ErbB protein present on the surface of a cell (e.g. via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of ErbB-encoding nucleic acid in the cell, e.g. via fluorescent in situ hybridization (FISH; see WO98/45479 published October, 1998), Southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study ErbB receptor overexpression by measuring shed antigen (e.g., ErbB extracellular domain) in a biological fluid such as serum (see, e.g., U.S. Pat. No. 4,933,294 issued Jun. 12, 1990; WO91/05264 published Apr. 18, 1991; U.S. Pat. No. 5,401,638 issued Mar. 28, 1995; and Sias et al. *J. Immunol. Methods* 132: 73≧80 (1990)). Aside from the above assays, various in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, e.g. a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, e.g. by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

The tumors overexpressing HER2 are rated by immunohistochemical scores corresponding to the number of copies of HER2 molecules expressed per cell, and can been determined biochemically: 0=0–10,000 copies/cell, 1+=at least about 200,000 copies/cell, 2+=at least about 500,000 copies/cell, 3+=at least about 2,000,000 copies/cell. Overexpression of HER2 at the 3+ level, which leads to ligand-independent activation of the tyrosine kinase (Hudziak et al., *Proc. Natl. Acad. Sci.* USA 84: 7159–7163 [1987]), occurs in approximately 30% of breast cancers, and in these patients, relapse-free survival and overall survival are diminished (Slamon et al., *Science* 244: 707–712 [1989]; Slamon et al., *Science* 235: 177–182 [1987]).

Conversely, a cancer which is "not characterized by overexpression of an ErbB receptor" is one which, in a diagnostic assay, does not express higher than normal levels of ErbB receptor compared to a noncancerous cell of the same tissue type.

A "hormone independent" cancer is one in which proliferation thereof is not dependent on the presence of a hormone which binds to a receptor expressed by cells in the cancer. Such cancers do not undergo clinical regression upon administration of pharmacological or surgical strategies that reduce the hormone concentration in or near the tumor. Examples of hormone independent cancers include androgen independent prostate cancer, estrogen independent breast cancer, endometrial cancer and ovarian cancer. Such cancers may begin as hormone dependent tumors and progress from a hormone-sensitive stage to a hormone-refractory tumor following anti-hormonal therapy.

The term "antibody" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., *Nature,* 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., *Nature,* 352:624–628 (1991) and Marks et al., *J. Mol. Biol.,* 222:581–597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci.* USA, 81:6851–6855 (1984)). Chimeric antibodies of interest herein include primatized antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g. Old World Monkey, Ape etc) and human constant region sequences.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

An "intact" antibody is one which comprises an antigen-binding variable region as well as a light chain constant domain (C_L) and heavy chain constant domains, C_H1, C_H2 and C_H3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

"Humanized" forms of non-human (e.g., rodent) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at

least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature* 321:522–525 (1986); Riechmann et al., *Nature* 332:323–329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593–596 (1992).

Humanized anti-ErbB2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and 4D5-8 (HERCEPTIN®) as described in Table 3 of U.S. Pat. No. 5,821,337 expressly incorporated herein by reference; humanized 520C9 (WO93/21319) and humanized 2C4 antibodies. The heavy chain and light chain of humanized antibody 2C4 are shown in FIGS. **1** and **2**, respectively.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc.

Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes". There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α, δ, ϵ, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells in summarized is Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457–92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. *PNAS (USA)* 95:652–656 (1998).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source thereof, e.g. from blood or PBMCs as described herein.

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see review M. in Daëron, *Annu. Rev. Immunol.* 15:203–234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457–92 (1991); Capel et al., *Immunomethods* 4:25–34 (1994); and de Haas et al., *J. Lab. Clin. Med.* 126:330–41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)).

"Complement dependent cytotoxicity" or "CDC" refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996), may be performed.

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

The term "variable", as used in connection with antibodies, refers to the fact that certain portions of the antibody variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. residues 24–34 (L1), 50–56 (L2) and 89–97 (L3) in the light chain variable domain and 31–35 (H1), 50–65 (H2) and 95–102 (H3) in the heavy chain variable domain; Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 26–32 (L1), 50–52 (L2) and 91–96 (L3) in the light chain variable domain and 26–32 (H1), 53–55 (H2) and 96–101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901–917 (1987)). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

An antibody "which binds" an antigen of interest, e.g. ErbB2 antigen, is one capable of binding that antigen with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting a cell expressing the antigen and/or for targeted delivery of a cytotoxic or other chemotherapeutic agent, such as a maytansinoid. Where the antibody is one which binds ErbB2, it will usually preferentially bind ErbB2 as opposed to other ErbB receptors, and may be one which does not significantly cross-react with other proteins such as EGFR, ErbB3 or ErbB4. In such embodiments, the extent of binding of the antibody to these non-ErbB2 proteins (e.g., cell surface binding to endogenous receptor) will be less than 10% as determined by fluorescence activated cell sorting (FACS) analysis or radioimmunoprecipitation (RIA). Sometimes, the anti-ErbB2 antibody will not significantly cross-react with the rat neu protein, e.g., as described in Schecter et al. *Nature* 312:513 (1984) and Drebin et al., *Nature* 312: 545–548 (1984).

Unless indicated otherwise, the expressions "monoclonal antibody 4D5", and "4D5 monoclonal antibody" refer to an antibody that has antigen binding residues of, or derived from, the murine 4D5 antibody. For example, the monoclonal antibody 4D5 may be murine monoclonal antibody 4D5 (ATCC CRL 10463) or a variant thereof, such as humanized antibody 4D5, possessing antigen binding amino acid residues of murine monoclonal antibody 4D5. Exemplary humanized 4D5 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®) as in U.S. Pat. No. 5,821,337, with huMAb4D5-8 (HERCEPTIN®) being a preferred humanized 4D5 antibody.

An antibody having a "biological characteristic" of a designated antibody, such as the monoclonal antibody designated 4D5, is one which possesses one or more of the biological characteristics of that antibody which distinguish it from other antibodies that bind to the same antigen (e.g. ErbB2). For example, an antibody with a biological characteristic of 4D5 may show growth inhibitory effect on ErbB2 overexpressing cells in a manner that is dependent on the ErbB2 expression level and/or bind the same epitope in the extracellular domain of ErbB2 as that bound by 4D5 (e.g. which blocks binding of monoclonal antibody 4D5 to ErbB2).

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially an ErbB expressing cancer cell either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of ErbB expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer,* Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13.

Examples of "growth inhibitory" antibodies are those which bind to ErbB2 and inhibit the growth of cancer cells overexpressing ErbB2. Preferred growth inhibitory anti-ErbB2 antibodies inhibit growth of SK-BR-3 breast tumor cells in cell culture by greater than 20%, and preferably greater than 50% (e.g. from about 50% to about 100%) at an antibody concentration of about 0.5 to 30 μg/ml, where the growth inhibition is determined six days after exposure of the SK-BR-3 cells to the antibody (see U.S. Pat. No. 5,677,171 issued Oct. 14, 1997). The SK-BR-3 cell growth inhibition assay is described in more detail in that patent and hereinbelow. The preferred growth inhibitory antibody is monoclonal antibody 4D5, e.g., humanized 4D5.

A molecule (e.g. antibody) which "induces cell death" is one which causes a viable cell to become nonviable. The cell is generally one which expresses the ErbB2 receptor, especially where the cell overexpresses the ErbB2 receptor. Preferably, the cell is a cancer cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic, prostate or bladder cancer cell. In vitro, the cell may be a SK-BR-3, BT474, Calu 3, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Cell death in vitro may be determined in the absence of complement and immune effector cells to distinguish cell death induced by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Thus, the assay for cell death may be performed using heat inactivated serum (i.e. in the absence of complement) and in the absence of immune effector cells. To determine whether the molecule is able to induce cell death, loss of membrane integrity as evaluated by uptake of propidium iodide (PI), trypan blue (see Moore et al. *Cytotechnology* 17:1–11 (1995)) or 7AAD can be assessed relative to untreated cells. Preferred cell death-

inducing antibodies are those which induce PI uptake in the PI uptake assay in BT474 cells. Examples of antibodies which induce cell death include anti-ErbB2 antibodies 7C2 and 7F3 (WO 98/17797, expressly incorporated herein by reference), including humanized and/or affinity matured variants thereof.

A molecule (e.g. antibody) which "induces apoptosis" is one which induces programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is usually one which overexpresses the ErbB2 receptor. Preferably the cell is a tumor cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic, prostate or bladder cancer cell. In vitro, the cell may be a SK-BR-3, BT474, Calu 3 cell, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the molecule which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an annexin binding assay using BT474 cells. Sometimes the pro-apoptotic molecule will be one which further blocks ErbB ligand activation of an ErbB receptor. In other situations, the molecule is one which does not significantly block ErbB ligand activation of an ErbB receptor. Further, the molecule may induce apoptosis, without inducing a large reduction in the percent of cells in S phase (e.g. one which only induces about 0–10% reduction in the percent of these cells relative to control). Examples of antibodies which induce apoptosis include anti-ErbB2 antibodies 7C2 and 7F3 (WO 98/17797, expressly incorporated herein by reference), including humanized and/or affinity matured variants thereof.

An antibody which "blocks" ligand activation of an ErbB receptor is one which reduces or prevents such activation as hereinabove defined, wherein the antibody is able to block ligand activation of the ErbB receptor substantially more effectively than monoclonal antibody 4D5, e.g. about as effectively as monoclonal antibodies 7F3 or 2C4 or Fab fragments thereof and preferably about as effectively as monoclonal antibody 2C4 or a Fab fragment thereof. For example, the antibody that blocks ligand activation of an ErbB receptor may be one which is about 50–100% more effective than 4D5 at blocking formation of an ErbB hetero-oligomer. Blocking of ligand activation of an ErbB receptor can occur by any means, e.g. by interfering with: ligand binding to an ErbB receptor, ErbB complex formation, tyrosine kinase activity of an ErbB receptor in an ErbB complex and/or phosphorylation of tyrosine kinase residue(s) in or by an ErbB receptor. Examples of antibodies which block ligand activation of an ErbB receptor include monoclonal antibodies 2C4 and 7F3 (which block HRG activation of ErbB2/ErbB3 and ErbB2/ErbB4 hetero-oligomers; and EGF, TGF-α, amphiregulin, HB-EGF and/or epiregulin activation of an EGFR/ErbB2 hetero-oligomer); and L26, L96 and L288 antibodies (Klapper et al. *Oncogene* 14:2099–2109 (1997)), which block EGF and NDF binding to T47D cells which express EGFR, ErbB2, ErbB3 and ErbB4. Humanized and/or affinity matured variants these and other antibodies within the definition are specifically included.

The term "epitope" is used to refer to binding sites for (monoclonal or polyclonal) antibodies on protein antigens.

Antibodies that bind to a certain epitope are identified by "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999. Competition assays are discussed below. According to the gene fragment expression assays, the open reading frame encoding the protein is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of the protein with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein in vitro, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled protein fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. The latter approach is suitable to define linear epitopes of about 5 to 15 amino acids.

An antibody binds "essentially the same epitope" as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two epitopes bind to identical or sterically overlapping epitopes are competition assays, which can be configured in all number of different formats, using either labeled antigen or labeled antibody. Usually, the antigen is immobilized on a 96-well plate, and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive or enzyme labels.

The "epitope 4D5" is the region in the extracellular domain of ErbB2 to which the antibody 4D5 (ATCC CRL 10463) binds. This epitope is close to the transmembrane domain of ErbB2, and extends from about residue 519 to about residue 625, inclusive within the ErbB2 extracellular domain sequence included in SEQ ID NO: 3, FIG. **4**. To screen for antibodies which bind to the 4D5 epitope, a routine cross-blocking assay such as that described in Harlow and Lane, supra, can be performed. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 4D5 epitope of ErbB2 (e.g. any one or more residues in the region from about residue 529 to about residue 625, inclusive in SEQ ID NO: 3).

The "epitope 3H4" is the region in the extracellular domain of ErbB2 to which the antibody 3H4 binds. This epitope includes residues from about 541 to about 599, inclusive, in the amino acid sequence of ErbB2 extracellular domain (see FIG. **4** and SEQ ID NO: 3).

The "epitope 7C2/7F3" is the region at the N terminus of the extracellular domain of ErbB2 to which the 7C2 and/or 7F3 antibodies (each deposited with the ATCC, see below) bind. To screen for antibodies which bind to the 7C2/7F3 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring

Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to establish whether the antibody binds to the 7C2/7F3 epitope on ErbB2 (e.g. any one or more of residues in the region from about residue 22 to about residue 53 of ErbB2; see FIG. **4**, and SEQ ID NO: 3).

A tumor which "does not respond, or responds poorly, to treatment with a monoclonal anti-ErbB antibody" does not show statistically significant improvement in response to anti-ErbB antibody treatment when compared to no treatment or treatment with placebo in a recognized animal model or a human clinical trial, or which responds to initial treatment with anti-ErbB antibodies but grows as treatment is continued. A particularly suitable animal model for testing the efficacy of anti-ErbB antibodies is the transgenic animal model disclosed herein, and illustrated in Example 3.

The terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

A "disorder" is any condition that would benefit from treatment of the present invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemias and lymphoid malignancies, in particular breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic, prostate or bladder cancer. A preferred disorder to be treated in accordance with the present invention is malignant tumor, such as breast cancer, that overexpresses an ErbB receptor (e.g. ErbB2 and/or EGFR), and does not respond or responds poorly to treatment with antibody to the receptor(s) that is/are overexpressed. A particularly preferred disorder is an ErbB2-overexpressing breast cancer that does not respond or responds poorly to HERCEPTIN® therapy.

The term "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

The term "objective response rate" refers to the number of treated individuals that respond to treatment as determined by a quantitative measure.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK7; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and

pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, e.g., Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions,* 14, pp. 375–382, 615th Meeting Belfast (1986) and Stella et al., "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery,* Borchardt et al., (ed.), pp. 247–267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

The term "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term also includes, as equivalents, analogs of either DNA or RNA made from nucleotide analogs, and as applicable, single (sense or antisense) and double-stranded polynucleotides. An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. The term "expression vector" includes plasmids, cosmids or phages capable of synthesizing the subject HER2 protein encoded by the respective recombinant gene carried by the vector. Preferred vectors are those capable of autonomous replication and/expression of nucleic acids to which they are linked. In the present specification, "plasmid" and "vector" are used interchangeably, as the plasmid is the most commonly used form of vector.

As used herein, the terms "transcriptional regulatory elements" and "transcriptional regulatory sequences" are used interchangeably and refer to nucleic acid, e.g. DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, enhancers, splicing signals and polyadenylation signals. These terms are intended to encompass all elements that promote or regulate transcription, including promoters, core elements required for basic interaction of RNA polymerase and transcription factors, upstream elements, enhancers, and response elements (Lewin, "Genes V" (Oxford University Press, Oxford) pages 847–873). Reference herein to the transcriptional regulatory elements of a gene or class of gene includes both all or an intact region of the naturally occurring transcriptional regulatory elements and modified forms of the transcriptional regulatory elements of the gene or group of genes. Such modified forms include rearrangements of the elements, deletions of some elements or extraneous sequences, and insertion of heterologous elements. The modular nature of transcriptional regulatory elements and the absence of position-dependence of the function of some regulatory elements such as enhancers make such modifications possible. Numerous techniques are available for dissecting the regulatory elements of genes to determine their location and function. Such information can be used to direct modification of the elements, if desired. It is preferred, however, that an intact region of the transcriptional regulatory elements of a gene be used.

The term "tissue-specific promoter" means a nucleotide sequence that serves as a promoter, i.e., regulates expression of a selected DNA sequence operably linked to the promoter, and which effects expression of the selected DNA sequence in specific cells of a tissue, such as cells of a mammary gland. In an illustrative embodiment, gene constructs utilizing mammary gland-specific promoters can be used to preferentially direct expression of a HER2 protein or protein fragment in the mammary gland tissue.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "transfection" refers to the introduction of a nucleic acid, e.g., an expression vector, into a recipient cell by nucleic acid-mediated gene transfer. "Transformation", as used herein, refers to a process in which a cell's genotype is changed as a result of the cellular uptake of exogenous DNA or RNA, and, for example, the transformed cell expresses a recombinant form of HER2.

As used herein, the term "transgene" refers to a nucleic acid sequence which is partly or entirely heterologous, i.e., foreign, to the transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion

results in a knockout). A transgene can be operably linked to one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that may be necessary for optimal expression of a selected nucleic acid.

Accordingly, the term "transgene construct" refers to a nucleic acid which includes a transgene, and (optionally) such other nucleic acid sequences as transcriptionally regulatory sequence, polyadenylation sites, replication origins, marker genes, etc., which may be useful in the general manipulation of the transgene for insertion in the genome of a host organism.

The term "transgenic" is used herein as an adjective to describe the property, for example, of an animal or a construct, of harboring a transgene. For instance, as used herein, a "transgenic organism" is any animal, preferably a non-human mammal, in which one or more of the cells of the animal contain heterologous nucleic acid introduced by way of human intervention, such as by trangenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. In the typical transgenic animals described herein, the transgene causes cells to express or overexpress a recombinant form of the subject HER2 proteins. The terms "founder line" and "founder animal" refer to those animals that are the mature product of the embryos to which the transgene was added, i.e., those animals that grew from the embryos into which DNA was inserted, and that were implanted into one or more surrogate hosts.

The terms "progeny" and "progeny of the transgenic animal" refer to any and all offspring of every generation subsequent to the originally transformed mammals. The term "non-human mammal" refers to all members of the class Mammalia except humans. "Mammal" refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as mouse, rat, rabbit, pig, sheep, goat, cattle and higher primates.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as the anti-ErbB2 antibodies disclosed herein and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

A "cardioprotectant" is a compound or composition which prevents or reduces myocardial dysfunction (i.e. cardiomyopathy and/or congestive heart failure) associated with administration of a drug, such as an anti-ErbB antibody or its maytansinoid conjugate, to a patient. The cardioprotectant may, for example, block or reduce a free-radical-mediated cardiotoxic effect and/or prevent or reduce oxidative-stress injury. Examples of cardioprotectants encompassed by the present definition include the iron-chelating agent dexrazoxane (ICRF-187) (Seifert et al. *The Annals of Pharmacotherapy* 28:1063–1072 (1994)); a lipid-lowering agent and/or anti-oxidant such as probucol (Singal et al. *J. Mol. Cell Cardiol.* 27:1055–1063 (1995)); amifostine (aminothiol 2-[(3-aminopropyl)amino]ethanethiol-dihydrogen phosphate ester, also called WR-2721, and the dephosphorylated cellular uptake form thereof called WR-1065) and S-3-(3-methylaminopropylamino)propylphosphorothioic acid (WR-151327), see Green et al. *Cancer Research* 54:738–741 (1994); digoxin (Bristow, M. R. In: Bristow M R, ed. *Drug-Induced Heart Disease.* New York: Elsevier 191–215 (1980)); beta-blockers such as metoprolol (Hjalmarson et al. *Drugs* 47:Suppl 4:31–9 (1994); and Shaddy et al. *Am. Heart J.* 129:197–9 (1995)); vitamin E; ascorbic acid (vitamin C); free radical scavengers such as oleanolic acid, ursolic acid and N-acetylcysteine (NAC); spin trapping compounds such as alpha-phenyl-tert-butyl nitrone (PBN); (Paracchini et al., *Anticancer Res.* 13:1607–1612 (1993)); selenoorganic compounds such as P251 (Elbesen); and the like.

2. Detailed Description

The present invention is based on results obtained in a novel murine HER2-transgenic tumor model in which HERCEPTIN® or the murine antibody 4D5 from which HERCEPTIN® was derived, had little effect on tumor growth. Using this model to test the efficacy of HERCEPTIN® and HERCEPTIN®-maytansinoid conjugates, it was surprisingly found that while the transplanted tumor obtained from such transgenic mice responded poorly to HERCEPTIN® treatment, the HERCEPTIN®-maytansinoid conjugates were highly efficacious.

Accordingly, the present invention is based on the use of anti-ErbB antibody-maytansinoid conjugates in the treatment of ErbB overexpressing tumors that do not respond well to anti-ErbB antibody and/or maytansinoid treatment.

A. Production of Anti-ErbB Antibodies

A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention. The production of antibodies will be illustrated with reference to anti-ErbB2 antibodies but it will be apparent for those skilled in the art that antibodies to other members of the ErbB receptor family can be produced and modified in a similar manner.

The ErbB2 antigen to be used for production of antibodies may be, e.g., a soluble form of the extracellular domain of ErbB2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing ErbB2 at their cell surface (e.g. NIH-3T3 cells transformed to overexpress ErbB2; or a carcinoma cell line such as SK-BR-3 cells, see Stancovski et al. *PNAS (USA)* 88:8691–8695 (1991)) can be used to generate antibodies. Other forms of ErbB2 useful for generating antibodies will be apparent to those skilled in the art.

(i) Polyclonal Antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet

hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with ⅕ to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal Antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59–103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); and Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51–63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al., *Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59–103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., *Curr. Opinion in Immunol.*, 5:256–262 (1993) and Plückthun, *Immunol. Revs.*, 130:151–188 (1992).

In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., *Nature*, 348:552–554 (1990). Clackson et al., *Nature*, 352:624–628 (1991) and Marks et al., *J. Mol. Biol.*, 222: 581–597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., *Bio/Technology*, 10:779–783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nuc. Acids. Res.*, 21:2265–2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy chain and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; and Morrison, et al., *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

(iii) Humanized Antibodies

Methods for humanizing non-human antibodies have been described in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature,* 321:522–525 (1986); Riechmann et al., *Nature,* 332:323–327 (1988); Verhoeyen et al., *Science,* 239:1534–1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims et al., *J. Immunol.,* 151:2296 (1993); Chothia et al., *J. Mol. Biol.,* 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al, *Proc. Natl. Acad. Sci.* USA, 89:4285 (1992); Presta et al., *J. Immunol.,* 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Example 1 below describes production of an exemplary humanized anti-ErbB2 antibody. The humanized antibody herein may, for example, comprise nonhuman hypervariable region residues incorporated into a human variable heavy domain and may further comprise a framework region (FR) substitution at a position selected from the group consisting of 69H, 71H and 73H utilizing the variable domain numbering system set forth in Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). In one embodiment, the humanized antibody comprises FR substitutions at two or all of positions 69H, 71H and 73H.

Various forms of the humanized antibody are contemplated. For example, the humanized antibody may be an antibody fragment, such as a Fab. Alternatively, the humanized antibody may be an intact antibody, such as an intact IgG1 antibody.

(iv) Human Antibodies

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci.* USA, 90:2551 (1993); Jakobovits et al., *Nature,* 362:255–258 (1993); Bruggermann et al., *Year in Immuno.,* 7:33 (1993); and U.S. Pat. Nos. 5,591,669, 5,589,369 and 5,545,807.

Alternatively, phage display technology (McCafferty et al., *Nature* 348:552–553 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564–571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., *Nature,* 352:624–628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., *J. Mol. Biol.* 222:581–597 (1991), or Griffith et al., *EMBO J.* 12:725–734 (1993). See, also, U.S. Pat. Nos. 5,565,332 and 5,573,905.

As discussed above, human antibodies may also be generated by in vitro activated B cells (see U.S. Pat. Nos. 5,567,610 and 5,229,275).

Human anti-ErbB2 antibodies are described in U.S. Pat. No. 5,772,997 issued Jun. 30, 1998 and WO 97/00271 published Jan. 3, 1997.

(v) Antibody Fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107–117 (1992); and Brennan et al., *Science,* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163–167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) Bispecific Antibodies

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the ErbB2 protein. Other such antibodies may combine an ErbB2 binding site with binding site(s) for EGFR, ErbB3 and/or ErbB4. Alternatively, an anti-ErbB2 arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the ErbB2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express ErbB2. WO 96/16673 describes a bispecific anti-ErbB2/anti-FcγRIII antibody and U.S. Pat. No. 5,837,234 discloses a bispecific anti-ErbB2/anti-FcγRI antibody. A bispecific anti-ErbB2/Fcα antibody is shown in WO98/02463. U.S. Pat. No. 5,821,337 teaches a bispecific anti-ErbB2/anti-CD3 antibody.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., *Nature,* 305:537–539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., *EMBO J.,* 10:3655–3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology,* 121:210 (1986).

According to another approach described in U.S. Pat. No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science,* 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli,* which can be chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.,* 175: 217–225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and

normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.*, 148(5):1547–1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444–6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., *J. Immunol.*, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al. *J. Immunol.* 147: 60 (1991).

(vii) Other Amino Acid Sequence Modifications

Amino acid sequence modification(s) of the anti-ErbB2 antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the anti-ErbB2 antibody are prepared by introducing appropriate nucleotide changes into the anti-ErbB2 antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the anti-ErbB2 antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the anti-ErbB2 antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the anti-ErbB2 antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells *Science*, 244: 1081-1085 (1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with ErbB2 antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed anti-ErbB2 antibody variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an anti-ErbB2 antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the anti-ErbB2 antibody molecule include the fusion to the N- or C-terminus of the anti-ErbB2 antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the anti-ErbB2 antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the anti-ErbB2 antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6–7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and human ErbB2. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

It may be desirable to modify the antibody of the invention with respect to effector function, e.g. so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., *J. Exp Med.* 176:1191–1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al. *Cancer Research* 53:2560–2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al. *Anti-Cancer Drug Design* 3:219–230 (1989).

To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Pat. No. 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

(viii) Glycosylation Variants

Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, *Chem. Immunol.* 65:111–128 [1997]; Wright and Morrison, *TibTECH* 15:26–32 [1997]). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., *Mol. Immunol.* 32:1311–1318 [1996]; Wittwe and Howard, *Biochem.* 29:4175–4180 [1990]), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, supra; Wyss and Wagner, *Current Opin. Biotech.* 7:409–416 [1996]). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. For example, it has been reported that in agalactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-CH2 space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (Malhotra et al., *Nature Med.* 1:237–243 [1995]). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL) (Boyd et al., *Mol. Immunol.* 32:1311–1318 [1996]), while selective removal of sialic acid residues using neuraminidase resulted in no loss of DMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana et al., *Mature Biotech.* 17:176–180 [1999]).

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (glycosylation pattern), the extent of glycosylation, etc.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites). Similarly, removal of glycosylation sites can be accomplished by amino acid alteration within the native glycosylation sites of the antibody.

The amino acid sequence is usually altered by altering the underlying nucleic acid sequence. Nucleic acid molecules encoding amino acid sequence variants of the anti-ErbB2 antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation

from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the anti-ErbB2 antibody.

The glycosylation (including glycosylation pattern) of antibodies may also be altered without altering the amino acid sequence or the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse et al., *J. Biol. Chem.* 272:9062–9070 [1997]). In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U.S. Pat. Nos. 5,047,335; 5,510,261 and 5,278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g. make defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, Mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo-β-galactosidase), elimination using harsh alkaline environment to release mainly O-linked structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides.

(viii) Screening for Antibodies with the Desired Properties

Techniques for generating antibodies have been described above. One may further select antibodies with certain biological characteristics, as desired.

For example, to identify growth inhibitory anti-ErbB2 antibodies, one may screen for antibodies which inhibit the growth of cancer cells which overexpress ErbB2. In one embodiment, the growth inhibitory antibody of choice is able to inhibit growth of SK-BR-3 cells in cell culture by about 20–100% and preferably by about 50–100% at an antibody concentration of about 0.5 to 30 μg/ml. To identify such antibodies, the SK-BR-3 assay described in U.S. Pat. No. 5,677,171 can be performed. According to this assay, SK-BR-3 cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillin streptomycin. The SK-BR-3 cells are plated at 20,000 cells in a 35 mm cell culture dish (2 mls/35 mm dish). 0.5 to 30 μg/ml of the anti-ErbB2 antibody is added per dish. After six days, the number of cells, compared to untreated cells are counted using an electronic COULTER™ cell counter. Those antibodies which inhibit growth of the SK-BR-3 cells by about 20–100% or about 50–100% may be selected as growth inhibitory antibodies.

To select for antibodies which induce cell death, loss of membrane integrity as indicated by, e.g., PI, trypan blue or 7AAD uptake may be assessed relative to control. The preferred assay is the PI uptake assay using BT474 cells. According to this assay, BT474 cells (which can be obtained from the American Type Culture Collection (Rockville, Md.)) are cultured in Dulbecco's Modified Eagle Medium (D-MEM):Ham's F-12 (50:50) supplemented with 10% heat-inactivated FBS (Hyclone) and 2 mM L-glutamine. (Thus, the assay is performed in the absence of complement and immune effector cells). The BT474 cells are seeded at a density of 3×10^6 per dish in 100×20 mm dishes and allowed to attach overnight. The medium is then removed and replaced with fresh medium alone or medium containing 10 μg/ml of the appropriate monoclonal antibody. The cells are incubated for a 3 day time period. Following each treatment, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged at 1200 rpm for 5 minutes at 4° C., the pellet resuspended in 3 ml ice cold Ca^{2+} binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) and aliquoted into 35 mm strainer-capped 12×75 tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 μg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce statistically significant levels of cell death as determined by PI uptake may be selected as cell death-inducing antibodies.

In order to select for antibodies which induce apoptosis, an annexin binding assay using BT474 cells is available. The BT474 cells are cultured and seeded in dishes as discussed in the preceding paragraph. The medium is then removed and replaced with fresh medium alone or medium containing 10 μg/ml of the monoclonal antibody. Following a three day incubation period, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged, resuspended in Ca^{2+} binding buffer and aliquoted into tubes as discussed above for the cell death assay. Tubes then receive labeled annexin (e.g. annexin V-FTIC) (1 μg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce statistically significant levels of annexin binding relative to control are selected as apoptosis-inducing antibodies.

In addition to the annexin binding assay, a DNA staining assay using BT474 cells is available. In order to perform this assay, BT474 cells which have been treated with the antibody of interest as described in the preceding two paragraphs are incubated with 9 μg/ml HOECHST 33342™ for 2 hr at 37° C., then analyzed on an EPICS ELITE™ flow cytometer (Coulter Corporation) using MODFIT LT™ software (Verity Software House). Antibodies which induce a change in the percentage of apoptotic cells which is 2 fold or greater (and preferably 3 fold or greater) than untreated cells (up to 100% apoptotic cells) may be selected as pro-apoptotic antibodies using this assay.

To identify an antibody which blocks ligand activation of an ErbB receptor, the ability of the antibody to block ErbB ligand binding to cells expressing the ErbB receptor (e.g. in conjugation with another ErbB receptor with which the ErbB receptor of interest forms an ErbB hetero-oligomer) may be determined. For example, cells naturally expressing, or transfected to express, ErbB receptors of the ErbB hetero-oligomer may be incubated with the antibody and then

exposed to labeled ErbB ligand. The ability of the anti-ErbB2 antibody to block ligand binding to the ErbB receptor in the ErbB hetero-oligomer may then be evaluated.

For example, inhibition of HRG binding to MCF7 breast tumor cell lines by anti-ErbB2 antibodies may be performed using monolayer MCF7 cultures on ice in a 24-well-plate format essentially as described in Example 1 below. Anti-ErbB2 monoclonal antibodies may be added to each well and incubated for 30 minutes. ^{125}I-labeled rHRG$\beta 1_{177-224}$ (25 pm) may then be added, and the incubation may be continued for 4 to 16 hours. Dose response curves may be prepared and an IC_{50} value may be calculated for the antibody of interest. In one embodiment, the antibody which blocks ligand activation of an ErbB receptor will have an IC_{50} for inhibiting HRG binding to MCF7 cells in this assay of about 50 nM or less, more preferably 10 nM or less. Where the antibody is an antibody fragment such as a Fab fragment, the IC_{50} for inhibiting HRG binding to MCF7 cells in this assay may, for example, be about 100 nM or less, more preferably 50nM or less.

Alternatively, or additionally, the ability of the anti-ErbB2 antibody to block ErbB ligand-stimulated tyrosine phosphorylation of an ErbB receptor present in an ErbB hetero-oligomer may be assessed. For example, cells endogenously expressing the ErbB receptors or transfected to expressed them may be incubated with the antibody and then assayed for ErbB ligand-dependent tyrosine phosphorylation activity using an anti-phosphotyrosine monoclonal (which is optionally conjugated with a detectable label). The kinase receptor activation assay described in U.S. Pat. No. 5,766,863 is also available for determining ErbB receptor activation and blocking of that activity by an antibody.

In one embodiment, one may screen for an antibody which inhibits HRG stimulation of p180 tyrosine phosphorylation in MCF7 cells. For example, the MCF7 cells may be plated in 24-well plates and monoclonal antibodies to ErbB2 may be added to each well and incubated for 30 minutes at room temperature; then rHRG$\beta 1_{177-244}$ may be added to each well to a final concentration of 0.2 nM, and the incubation may be continued for 8 minutes. Media may be aspirated from each well, and reactions may be stopped by the addition of 100 μl of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 μl) may be electrophoresed on a 4–12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (at 1 μg/ml) immunoblots may be developed, and the intensity of the predominant reactive band at M_r~180,000 may be quantified by reflectance densitometry. The antibody selected will preferably significantly inhibit HRG stimulation of p180 tyrosine phosphorylation to about 0–35% of control in this assay. A dose-response curve for inhibition of HRG stimulation of p180 tyrosine phosphorylation as determined by reflectance densitometry may be prepared and an IC_{50} for the antibody of interest may be calculated. In one embodiment, the antibody which blocks ligand activation of an ErbB receptor will have an IC_{50} for inhibiting HRG stimulation of p180 tyrosine phosphorylation in this assay of about 50 nM or less, more preferably 10 nM or less. Where the antibody is an antibody fragment such as a Fab fragment, the IC_{50} for inhibiting HRG stimulation of p180 tyrosine phosphorylation in this assay may, for example, be about 100 nM or less, more preferably 50 nM or less.

One may also assess the growth inhibitory effects of the antibody on MDA-MB-175 cells, e.g. essentially as described in Schaefer et al. *Oncogene* 15:1385–1394 (1997). According to this assay, MDA-MB-175 cells may treated with an anti-ErbB2 monoclonal antibody (10 μg/mL) for 4 days and stained with crystal violet. Incubation with an anti-ErbB2 antibody may show a growth inhibitory effect on this cell line similar to that displayed by monoclonal antibody 2C4. In a further embodiment, exogenous HRG will not significantly reverse this inhibition. Preferably, the antibody will be able to inhibit cell proliferation of MDA-MB-175 cells to a greater extent than monoclonal antibody 4D5 (and optionally to a greater extent than monoclonal antibody 7F3), both in the presence and absence of exogenous HRG.

In one embodiment, the anti-ErbB2 antibody of interest may block heregulin dependent association of ErbB2 with ErbB3 in both MCF7 and SK-BR-3 cells as determined in a co-immunoprecipitation experiment substantially more effectively than monoclonal antibody 4D5, and preferably substantially more effectively than monoclonal antibody 7F3.

To screen for antibodies which bind to an epitope on ErbB2 bound by an antibody of interest, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual,* Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, or additionally, epitope mapping can be performed by methods known in the art (see, e.g. FIGS. 1A and 1B herein).

The results obtained in the cell-based assays described above can then be followed by testing in animal, e.g. murine, models, and human clinical trials. In particular, the inability or limited ability of an antibody to treat ErbB2 overexpressing tumors can be demonstrated in the transgenic mouse model disclosed in the present application as described in the Examples below.

B. Anti-ErbB Antibody-maytansinoid Conjugates (Immunoconjugates)

Anti-ErbB antibody-maytansinoid conjugates are prepared by chemically linking an anti-ErbB antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed, for example, in U.S. Pat. No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytansinoids are maytansinol and maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

There are many linking groups known in the art for making antibody-maytansinoid conjugates, including, for example, those disclosed in U.S. Pat. No. 5,208,020 (e.g., at column 7, lines 55-67 and at column 8, lines 1-14), or EP Patent 0 425 235 B1, and Chari et al. *Cancer Research* 52:127–131(1992). The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents, disulfide and thioether groups being preferred. For example, for a compound as illustrated in FIG. 3, "R" may be SH or may be SSR_1, where R_1 represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl or heterocyclic.

In addition, there are many possible sites within the antibody molecule for linking maytansinoid to the antibody. For example, in one embodiment HERCEPTIN® can be linked to the maytansinoid at lysine 13 in the light chain, at lysine 32 in the heavy chain, at lysine 26 in both Fab fragments and at lysine 38 in the Fc fragment.

Conjugates of the antibody and maytansinoid may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particularly preferred coupling agents include N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (Carlsson et al., *Biochem. J.* 173:723–737 [1978]) and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage.

The linker may be attached to the maytansinoid molecule at various positions, depending on the type of the link. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. The reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In a preferred embodiment, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

C. Pharmaceutical Formulations

Therapeutic formulations of the antibody-maytansinoid conjugates used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Preferred lyophilized anti-ErbB2 antibody formulations are described in WO 97/04801, expressly incorporated herein by reference.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide antibodies or antibody-maytansinoid conjugates which bind to EGFR, ErbB2 (e.g. an antibody which binds a different epitope on ErbB2), ErbB3, ErbB4, or vascular endothelial factor (VEGF) in the one formulation. Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

In one embodiment, the formulation comprises 5 mg/ml HERCEPTIN®-DM1, 100 mg/ml sucrose, 0.1% polysorbate 20 and 10 mM sodium succinate at pH 5.0.

D. Treatment with the Anti-ErbB2 Antibody-maytansinoid Conjugates

It is contemplated that, according to the present invention, the anti-ErbB2 antibody-maytansinoid conjugates may be used to treat various diseases or disorders. Exemplary conditions or disorders include benign or malignant tumors; leukemias and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic disorders.

Generally, the disease or disorder to be treated is cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

The cancer will comprise ErbB-expressing cells, such that an anti-ErbB antibody herein is able to bind to the cancer, and will be typically characterized by overexpression of the ErbB receptor. In a preferred embodiment, the cancer comprises ErbB2-expressing cells, even more preferably, cells

which are characterized by overexpression of the ErbB2 receptor. To determine ErbB, e.g. ErbB2 expression in the cancer, various diagnostic/prognostic assays are available. In one embodiment, ErbB2 overexpression may be analyzed by IHC, e.g. using the HERCEPTEST® (Dako). Parrafin embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a ErbB2 protein staining intensity criteria as follows:

Score 0

no staining is observed or membrane staining is observed in less than 10% of tumor cells.

Score 1+

a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.

Score 2+

a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

Score 3+

a moderate to strong complete membrane staining is observed in more than 10% of the tumor cells.

Those tumors with 0 or 1+ scores for ErbB2 overexpression assessment may be characterized as not overexpressing ErbB2, whereas those tumors with 2+ or 3+ scores may be characterized as overexpressing ErbB2.

Alternatively, or additionally, fluorescence in situ hybridization (FISH) assays such as the INFORM™ (sold by Ventana, Ariz.) or PATHVISION™ (Vysis, Ill.) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of ErbB2 overexpression in the tumor. In comparison with IHC assay, the FISH assay, which measures her2 gene amplification, seems to correlate better with response of patients to treatment with HERCEPTIN®, and is currently considered to be the preferred assay to identify patients likely to benefit from HERCEPTIN® treatment or treatment with the immunoconjugates of the present invention.

In one embodiment, the cancer will be one which expresses (and may overexpress) EGFR. Examples of cancers which may express/overexpress EGFR include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

Preferably, the immunoconjugates of the present invention and/or ErbB, e.g. ErbB2 or EGFR protein to which they are bound are internalized by the cell, resulting in increased therapeutic efficacy of the immunoconjugate in killing the cancer cell to which they bind. In a preferred embodiment, the cytotoxic agent (maytansinoid) targets or interferes with nucleic acid in the cancer cell.

The treatment of the present invention targets ErbB overexpressing tumors that do not respond, or respond poorly, to treatment with an unconjugated anti-ErbB antibody. Such patients might have received prior treatment with an anti-ErbB antibody not conjugated to a maytansinoid moiety, where the prior treatment either did not result in significant improvement, or resulted in transient response. Prior treatment of any particular patient with an unconjugated anti-ErbB antibody is, however, not a prerequisite of identifying patients who are candidates for treatment in accordance with the present invention. An ordinary skilled physician can readily identify patients who are expected to benefit from treatment with the immunoconjugates of the present invention based on publicly available clinical data and his or her own experience. Treatment of mammals, and in particular human patients, with or without prior treatment with an (unconjugated) anti-ErbB antibody is specifically within the scope of the present invention.

The anti-ErbB antibody-maytansinoid conjugates are administered to a mammal, preferably to a human patient in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the antibody is preferred.

Other therapeutic regimens may be combined with the administration of the anti-ErbB antibody-maytansinoid conjugates. The combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

In one preferred embodiment, the patient is treated with two or more different anti-ErbB antibodies, at least one of which is in the form of a maytansinoid conjugate. For example, the patient may be treated with a first anti-ErbB2 antibody-maytansinoid conjugate in which the antibody is growth inhibitory (e.g. HERCEPTIN®), and a second anti-ErbB2 antibody or antibody-immunoconjugate, e.g. an antibody-maytansinoid conjugate which blocks ligand activation of an ErbB receptor (e.g. 2C4 or a humanized and/or affinity matured variant thereof) or induces apoptosis of an ErbB2-overexpressing cell (e.g. 7C2, 7F3 or humanized variants thereof). In another embodiment, the treatment involves the administration of antibodies that specifically bind two or more different ErbB receptors, such as, for example, ErbB2 and EGFR receptors, where at least one of the anti-ErbB antibodies is administered as a maytansinoid conjugate. Preferably such combined therapy results in a synergistic therapeutic effect.

It may also be desirable to combine administration of the anti-ErbB antibody-maytansinoid conjugates, with administration of an antibody directed against another tumor-associated antigen, which is not member of the ErbB family of receptors. The other antibody in this case may, for example, bind to vascular endothelial growth factor (VEGF), and may be in the form of a maytansinoid conjugate, or another immunoconjugate.

In one embodiment, the treatment of the present invention involves the combined administration of an anti-ErbB2 antibody-maytansinoid conjugate (or conjugates) and one or more chemotherapeutic agents or growth inhibitory agents, including coadministration of cocktails of different chemotherapeutic agents. Preferred chemotherapeutic agents include taxanes (such as paclitaxel and doxetaxel) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md. (1992).

In a preferred embodiment, the treatment is initiated with an anti-ErbB antibody-maytansinoid conjugate, followed by maintenance treatment with an unconjugated or 'naked' anti-ErbB antibody. This strategy may eliminate or reduce tumor cells resistant to the naked antibody in the initial round because of the ability of the antibody-DM1 conjugate to effectively kill such tumor cells.

The antibody-maytansinoid conjugates may be combined with an anti-hormonal compound; e.g., an anti-estrogen compound such as tamoxifen; an anti-progesterone such as onapristone (see, EP 616 812); or an anti-androgen such as flutamide, in dosages known for such molecules. Where the cancer to be treated is hormone independent cancer, the patient may previously have been subjected to anti-hormonal therapy and, after the cancer becomes hormone independent, the anti-ErbB2 antibody (and optionally other agents as described herein) may be administered to the patient.

Sometimes, it may be beneficial to also coadminister a cardioprotectant (to prevent or reduce myocardial dysfunction associated with the therapy) or one or more cytokines to the patient. In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

Suitable dosages for any of the above coadministered agents are those presently used and may be lowered due to the combined action (synergy) of the agent and anti-ErbB2 antibody.

For the prevention or treatment of disease, the appropriate dosage of antibody-maytansinoid conjugates will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody-maytansinoid conjugate is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1–20 mg/kg) of antibody-maytansinoid conjugate is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. A preferred dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the anti-ErbB2 antibody-maytansinoid conjugate. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Based on the data disclosed herein, it is anticipated that one useful dosing protocol may entail approximately weekly (or less frequent) administration of the anti-ErbB antibody-maytansinoid conjugate where each dose of the conjugate is about 0.2–10 mg/kg, preferably about 1–3 mg/kg of the conjugate (e.g. where there are 1 to about 10, preferably about 3–4, maytansinoid molecules conjugated to each antibody molecule). From about 2–10, preferably about 4–6, dosages of the conjugate may be administered to the patient approximately every week.

In a preferred embodiment, the patients are treated initially with anti-ErbB-maytansinoid conjugate followed by therapy with unconjugated anti-ErbB antibody. Preferably, the anti-ErbB antibody in the conjugate and the unconjugated antibody are the same antibody. For example, treatment could be initiated with weekly injections of HERCEPTIN®-DM1 at about 0.5–5 mg/kg, preferably at about 1–3 mg/kg for 4–6 weeks, with the option of repeating this treatment. Patients can then be rolled over to conventional HERCEPTIN® therapy, which typically consists of treatment with a 4 mg/kg initial dose of HERCEPTIN®, followed by weekly treatment with a maintenance dose of 2 mg/kg. However, the 4 mg/kg initial dose may be omitted, with therapy going straight to the 2 mg/kg maintenance dose.

E. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-ErbB2 antibody-maytansinoid conjugate. In one embodiment the container is a 10 cc vial containing 10 mL of a solution comprising HERCEPTIN®-DM1.

The label or package insert indicates that the composition is used for treating the condition of choice, such as cancer. In one embodiment, the label or package inserts indicates that the composition comprising the antibody which binds ErbB2 can be used to treat cancer which expresses an ErbB receptor selected from the group consisting of epidermal growth factor receptor (EGFR), ErbB2, ErbB3 and ErbB4, preferably EGFR. In addition, the label or package insert may indicate that the patient to be treated is one having cancer characterized by excessive activation of an ErbB receptor selected from EGFR, ErbB2, ErbB3 or ErbB4. For example, the cancer may be one which overexpresses one of these receptors and/or which overexpresses an ErbB ligand (such as TGF-α). The label or package insert may also indicate that the composition can be used to treat cancer, wherein the cancer is not characterized by overexpression of the ErbB2 receptor. For example, whereas the present package insert for HERCEPTIN® indicates that the antibody is used to treat patients with metastatic breast cancer whose tumors overexpress the ErbB2 protein, the package insert herein may indicate that the antibody or composition is used to treat cancer that does not respond, or respond poorly, to treatment with HERCEPTIN®. In other embodiments, the package insert may indicate that the antibody-maytansinoid conjugate or composition can be used also to treat hormone independent cancer, prostate cancer, colon cancer or colorectal cancer.

Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a maytansinoid conjugate of a first antibody which binds ErbB2 and inhibits growth of cancer cells which overexpress ErbB2; and (b) a second container with a composition contained therein, wherein the composition comprises a second antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor, or a conjugate of this second antibody with a maytansinoid. The article of manufacture in this embodiment of the invention may further comprises a package insert indicating that the first and second compositions can be used to treat cancer.

Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Further details of the invention are illustrated in the following non-limiting examples.

EXAMPLE 1

Production Characterization and Humanization of Anti-ErbB2 Monoclonal Antibody 4D5

The murine monoclonal antibody 4D5 which specifically binds the extracellular domain of ErbB2 was produced as described in Fendly et al., *Cancer Research* 50:1550–1558 (1990). Briefly, NIH 3T3/HER2-3_{400} cells (expressing approximately 1×10^5 ErbB2 molecules/cell) produced as described in Hudziak et al *Proc. Natl. Acad. Sci. (USA)* 84:7158–7163 (1987) were harvested with phosphate buffered saline (PBS) containing 25 mM EDTA and used to immunize BALB/c mice. The mice were given injections i.p. of 10^7 cells in 0.5 ml PBS on weeks 0, 2, 5 and 7. The mice with antisera that immunoprecipitated ^{32}P-labeled ErbB2 were given i.p. injections of a wheat germ agglutinin-Sepharose (WGA) purified ErbB2 membrane extract on weeks 9 and 13. This was followed by an i.v. injection of 0.1 ml of the ErbB2 preparation and the splenocytes were fused with mouse myeloma line X63-Ag8.653. Hybridoma supernatants were screened for ErbB2-binding by ELISA and radioimmunoprecipitation.

Epitope Mapping and Characterization

The ErbB2 epitope bound by monoclonal antibody 4D5 was determined by competitive binding analysis (Fendly et al. *Cancer Research* 50:1550–1558 (1990)). Cross-blocking studies were done by direct fluorescence on intact cells using the PANDEX™ Screen Machine to quantitate fluorescence. The monoclonal antibody was conjugated with fluorescein isothiocyanate (FITC), using established procedures (Wofsy et al. *Selected Methods in Cellular Immunology*, p. 287, Mishel and Schiigi (eds.) San Francisco: W. J. Freeman Co. (1980)). Confluent monolayers of NIH 3T3/HER2-3_{400} cells were trypsinized, washed once, and resuspended at 1.75×10^6 cell/ml in cold PBS containing 0.5% bovine serum albumin (BSA) and 0.1% NaN_3. A final concentration of 1% latex particles (IDC, Portland, Oreg.) was added to reduce clogging of the PANDEX™ plate membranes. Cells in suspension, 20 µl, and 20 µl of purified monoclonal antibodies (100 µg/ml to 0.1 µg/ml) were added to the PANDEX™ plate wells and incubated on ice for 30 minutes. A predetermined dilution of the FITC-labeled monoclonal antibody in 20 µl was added to each well, incubated for 30 minutes, washed, and the fluorescence was quantitated by the PANDEX™. Monoclonal antibodies were considered to share an epitope if each blocked binding of the other by 50% or greater in comparison to an irrelevant monoclonal antibody control. In this experiment, monoclonal antibody 4D5 was assigned epitope I (amino acid residues from about 529 to about 625, inclusive within the ErbB2 extracellular domain (see SEQ ID NO: 3).

The growth inhibitory characteristics of monoclonal antibody 4D5 were evaluated using the breast tumor cell line, SK-BR-3 (see Hudziak et al. *Molec. Cell. Biol.* 9(3):1165–1172 (1989)). Briefly, SK-BR-3 cells were detached by using 0.25% (vol/vol) trypsin and suspended in complete medium at a density of 4×10^5 cells per ml. Aliquots of 100 µl (4×10^4 cells) were plated into 96-well microdilution plates, the cells were allowed to adhere, and 100 µl of media alone or media containing monoclonal antibody (final concentration 5 µg/ml) was then added. After 72 hours, plates were washed twice with PBS (pH 7.5), stained with crystal violet (0.5% in methanol), and analyzed for relative cell proliferation as described in Sugarman et al. *Science* 230:943–945 (1985). Monoclonal antibody 4D5 inhibited SK-BR-3 relative cell proliferation by about 56%.

Monoclonal antibody 4D5 was also evaluated for its ability to inhibit HRG-stimulated tyrosine phosphorylation of proteins in the M_r 180,000 range from whole-cell lysates of MCF7 cells (Lewis et al. *Cancer Research* 56:1457–1465 (1996)). MCF7 cells are reported to express all known ErbB receptors, but at relatively low levels. Since ErbB2, ErbB3, and ErbB4 have nearly identical molecular sizes, it is not possible to discern which protein is becoming tyrosine phosphorylated when whole-cell lysates are evaluated by Western blot analysis. However, these cells are ideal for HRG tyrosine phosphorylation assays because under the assay conditions used, in the absence of exogenously added HRG, they exhibit low to undetectable levels of tyrosine phosphorylation proteins in the M_r 180,000 range.

MCF7 cells were plated in 24-well plates and monoclonal antibodies to ErbB2 were added to each well and incubated for 30 minutes at room temperature; then rHRGβ$1_{177-244}$ was added to each well to a final concentration of 0.2 nM, and the incubation was continued for 8 minutes. Media was carefully aspirated from each well, and reactions were stopped by the addition of 100 µl of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 µl) was electrophoresed on a 4–12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (4G10, from UBI, used at 1 µg/ml) immunoblots were developed, and the intensity of the predominant reactive band at M_r—180,000 was quantified by reflectance densitometry, as described previously (Holmes et al. *Science* 256:1205–1210 (1992); Sliwkowski et al. *J. Biol. Chem.* 269:14661–14665 (1994))

Monoclonal antibody 4D5 significantly inhibited the generation of a HRG-induced tyrosine phosphorylation signal at M_r 180,000. In the absence of HRG, but was unable to stimulate tyrosine phosphorylation of proteins in the M_r 180,000 range. Also, this antibody does not cross-react with EGFR (Fendly et al. *Cancer Research* 50:1550–1558 (1990)), ErbB3, or ErbB4. Monoclonal antibody 4D5 was able to block HRG stimulation of tyrosine phosphorylation by ~50%.

The growth inhibitory effect of monoclonal antibody 4D5 on MDA-MB-175 and SK-BR-3 cells in the presence or absence of exogenous rHRGβ1 was assessed (Schaefer et al. *Oncogene* 15:1385–1394 (1997)). ErbB2 levels in MDA-MB-175 cells are 4–6 times higher than the level found in normal breast epithelial cells and the ErbB2-ErbB4 receptor is constitutively tyrosine phosphorylated in MDA-MB-175 cells. Monoclonal antibody 4D5 was able to inhibit cell proliferation of MDA-MB-175 cells, both in the presence and absence of exogenous HRG. Inhibition of cell proliferation by 4D5 is dependent on the ErbB2 expression level (Lewis et al. *Cancer Immunol. Immunother.* 37:255–263 (1993)). A maximum inhibition of 66% in SK-BR-3 cells could be detected. However this effect could be overcome by exogenous HRG.

Humanization

The murine monoclonal antibody 4D5 was humanized, using a novel "gene conversion mutagenesis" strategy, as described in U.S. Pat. No. 5,821,337, the entire disclosure of which is hereby expressly incorporated by reference. The humanized monoclonal antibody 4D5 used in the following experiments is designated huMAb4D5-8. This antibody is of IgG1 isotype.

EXAMPLE 2

HERCEPTIN®-DM1 Conjugates

1. Purification of HERCEPTIN®

HERCEPTIN® (huMAb4D5-8, rhuMAb HER2, U.S. Pat. No. 5,821,337) (1 vial containing 440 mg antibody) was dissolved in 50 mL MES buffer (25 mM MES, 50 mM NaCl, pH 5.6). The sample was loaded on a cation exchange column (Sepharose S, 15 cm×1.7 cm) that had been equilibrated in the same buffer. The column was then washed with the same buffer (5 column volumes). HERCEPTIN® was eluted by raising the NaCl concentration of the buffer to 200 mM. Fractions containing the antibody were pooled, diluted to 10 mg/mL, and dialyzed into a buffer containing 50 mm potassium phosphate, 50 mM NaCl, 2 mM EDTA, pH 6.5.

2. Modification of HERCEPTIN® with SPP

The purified HERCEPTIN® antibody was modified with N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to introduce dithiopyridyl groups. The antibody (376.0 mg, 8 mg/mL) in 44.7 mL of 50 mM potassium phosphate buffer (pH 6.5) containing NaCl (50 mM) and EDTA (1 mM) was treated with SPP (5.3 molar equivalents in 2.3 mL ethanol). After incubation for 90 minutes under argon at ambient temperature, the reaction mixture was gel filtered through a Sephadex G25 column equilibrated with 35 mM sodium citrate, 154 mM NaCl, 2 mM EDTA. Antibody containing fractions were pooled and assayed. The degree of modification of the antibody was determined as described above. Recovery of the modified antibody (HERCEPTIN®-SPP-Py) was 337 mg (89.7%) with 4.5 releasable 2-thiopyridine groups linked per antibody.

3. Conjugation of HERCEPTIN®-SPP-Py with DM1

The modified antibody (337.0 mg, 9.5 μmols of releasable 2-thiopyridine groups) was diluted with the above 35 mM sodium citrate buffer, pH 6.5, to a final concentration of 2.5 mg/mL. DM1 (1.7 equivalents, 16.1 μmols) in 3.0 mM dimethylacetamide (DMA, 3% v/v in the final reaction mixture) was then added to the antibody solution. The structure of DM1 is shown in FIG. **3**, where the nature of the "R" group is not critical and can be occupied, for example, by a variety of groups capable of forming a chemical bond with a linker. DM1 used in the present reaction was stored as an S—S form, which is more stable, and was reduced to the SH form for conjugation with the HERCEPTIN® antibody. The reaction proceeded at ambient temperature under argon for 20 hours. The structure of HERCEPTIN®-DM1 conjugates is illustrated in FIG. **4**.

The reaction was loaded on a Sephacryl S300 gel filtration column (5.0 cm×90.0 cm, 1.77 L) equilibrated with 35 mM sodium citrate, 154 mM NaCl, pH 6.5. The flow rate was 5.0 mL/min and 65 fractions (20.0 mL each) were collected. A major peak centered around fraction No. 47 (FIG. **5**). The major peak comprises monomeric HERCEPTIN®-DM1. Fractions 44–51 were pooled and assayed. The number of DM1 drug molecules linked per antibody molecule was determined by measuring the absorbance at 252 nm and 280 nm, and found to be 3.7 drug molecules per antibody molecule.

4. Anti-proliferative Effect of HERCEPTIN®-DM1 Conjugate in vitro

SK-BR3 cells, which express 3+ level of HER2 on cell surface, were treated with HERCEPTIN®, HERCEPTIN®-DM1 conjugate, control mAb RITUXAN® or RITUXAN®-DM1 conjugates, and the effect of these treatments on cell proliferation was monitored. As shown in FIG. **6**, the extent of cell growth inhibition by treatment with HERCEPTIN®-DM1 was dramatically more pronounced than that with HERCEPTIN®, while the control RITUXAN® antibody did not inhibit cell growth. Although the RITUXAN®-DM1 did inhibit cell growth, it did so only at high concentrations. For example, the RITUXAN®-DM1 conjugate did not inhibit growth at concentration up to 1 μg/ml. In contrast, the HERCEPTIN®-DM1 conjugate was highly potent and significantly inhibited cell growth starting from 0.01 μg/ml and reaching a plateau at 0.1 μg/ml. The RITUXAN®-DM1 conjugate required 100 times higher concentration to achieve the same level of cell growth inhibition as HERCEPTIN®-DM1 conjugate. This is also reflected in a 100-fold difference in IC_{50} value, concentration required to inhibit cell growth by 50%, of the respective conjugates.

EXAMPLE 3

Transgenic Animals

In order to improve the clinical activity of HERCEPTIN®, a transgenic HER2 mouse model was developed in which novel HER2-directed therapies could be tested preclinically. Tumors arise readily in transgenic mice that express a mutationally activated form of neu, the rat homolog of HER2, but the HER2 that is overexpressed in breast cancers is not mutated and tumor formation is much less robust in transgenic mice that overexpress nonmutated HER2 (Webster et al., *Semin. Cancer Biol.* 5: 69–76 [1994]). To improve tumor formation with nonmutated HER2, a strategy was used to further enhance overexpression of nonmutated HER2 in a transgenic mouse.

Any promoter that promotes expression of HER2 in epithelial cells in the mouse mammary gland can be used in the disclosed constructs. Many of the milk protein genes are transcribed by promoter/enhancer elements that are specifically active in mammary glands. Milk protein genes include those genes encoding caseins (α-S_1 and β), β-lactoglobulin, α-lactalbumin, and whey acidic protein. The ovine β-lactoglobulin promoter is well characterized and widely used in the art (Whitelaw et al., *Biochem J.* 286: 31–39, [1992]). However, similar fragments of promoter DNA from other species are also suitable. A preferred promoter is the promoter derived from the Long Terminal Repeat (LTR) of the Mouse Mammary Tumor Virus (MMTV). A HER2 transgene construct of the present invention was generated using the MMTV LTR promoter.

To improve tumor formation with nonmutated HER2, we have made transgenic mice using a HER2 cDNA plasmid in which an upstream ATG was deleted in order to prevent initiation of translation at such upstream ATG codons, which would otherwise reduce the frequency of translation initiation from the downstream authentic initiation codon of HER2 (for example, see Child et al., *J. Biol. Chem.* 274: 24335–24341 [1999]). Additionally, a chimeric intron was added to the 5' end, which should also enhance the level of expression as reported earlier (Neuberger and Williams,

Nucleic Acids Res. 16: 6713 [1988]; Buchman and Berg, *Mol. Cell. Biol.* 8: 4395 [1988]; Brinster et al., *Proc. Natl. Acad. Sci. USA* 85: 836 [1988]). The chimeric intron was derived from a Promega vector, pCI-neo mammalian expression vector (bp 890–1022). The cDNA 3'-end is flanked by human growth hormone exons 4 and 5, and polyadenylation sequences. Moreover, FVB mice were used because this strain is more susceptible to tumor development. The promoter from MMTV-LTR was used to ensure tissue-specific HER2 expression in the mammary gland. Animals were fed the AIN 76A diet in order to increase susceptibility to tumor formation (Rao et al., *Breast Cancer Res. and Treatment* 45: 149–158 [1997]). The nucleotide sequence of this transgene plasmid construct (SEQ ID NO: 1) is shown in FIG. 7.

Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Taconic (Germantown, N.Y.). Many strains are suitable, but FVB female mice are preferred because of their higher susceptibility to tumor formation. FVB males were used for mating and vasectomized CD. 1 studs were used to stimulate pseudopregnancy. Vasectomized mice can be obtained from any commercial supplier. Founders were bred with either FVB mice or with 129/BL6×FVB p53 heterozygous mice. The mice with heterozygosity at p53 allele were used to potentially increase tumor formation. However, this has proven unecessary. Therefore, some F1 tumors are of mixed strain. Founder tumors are FVB only. Six founders were obtained with some developing tumors without having litters.

EXAMPLE 4

HER2 Transgenic Mouse as a Tumor Model to Evaluate HER2-directed Therapies

Mammary gland biopsies of one founder transgenic mouse made as described in Example 3, showed 3+ expression of HER2, as determined by immunohistochemical staining, at about 2 months of age. The amount of HER2 extracellular domain (ECD) shed into serum was measured and found to be about 1.2 ng/ml (Huang et al., supra). This mouse subsequently developed a mammary tumor at 5 months of age, after bearing 4 litters. The tumor was surgically resected under aseptic conditions and minced into small pieces, 2 mm^3, which were then transplanted into the mammary fat pad of wild-type FVB female mice. As can be seen in FIG. 8A, the amount of HER2 ECD shed into serum increased over time following transplant and was found to be directly proportional to the weight of the tumor that developed (FIG. 8B). Tumors developed in 22 of 31 recipient mice, with a latency of 5 weeks. With subsequent passage, tumors developed with shorter latency and grew more rapidly, and tumor incidence increased to >95% of recipients. HER2 expression, as determined by immunohistochemical staining, was 3+ but heterogeneous in the primary tumor, but became uniformly 3+ after the first passage.

Treatment of tumor-bearing mice with HERCEPTIN® or 4D5, the murine antibody from which humanized HERCEPTIN® was derived, had only a modest effect on the growth of the transplanted tumors (FIG. 9). HER2 expression was 3+ in tumors that grew during HERCEPTIN® or 4D5 therapy, indicating that there was no selection of HER2-negative tumors. Moreover, as can be seen in FIG. 10, cy3-HERCEPTIN® was detected decorating tumor cells after injection into tumor-bearing mice, indicating that the lack of efficacy was not due to failure of the antibody to access the tumor. In addition, HER2 appears to be activated in the tumor cells, as evidenced by the binding of an anti-tyrosine phosphorylated HER2 antibody (FIG. 10).

Based on the persistent expression of HER2 and the failure of this tumor model to respond to HERCEPTIN®, a novel approach was tested, using HERCEPTIN® conjugated to maytansinoid DM1 as described in Example 3. FIG. 9 shows that the HERCEPTIN®-DM1 conjugate has dramatic anti-tumor activity in this model. RITUXAN®, an unrelated anti-CD20 monoclonal antibody, was used as a negative control for these studies. There was little response to HERCEPTIN® compared to the control antibody, RITUXAN®, but there was striking anti-tumor activity of the maytansinoid conjugate of HERCEPTIN®. As shown in FIG. 9, all of the mice treated with HERCEPTIN®-maytansinoid showed striking shrinkage of their tumors, though none of the tumors disappeared. After approximately 4 weeks, tumors began to regrow. Five animals were sacrificed at this time. Their tumors were found to express HER2 at 3+ levels. Thus, there was no selection for HER2-negative tumors. Based on this observation, the remaining 3 mice were treated with HERCEPTIN®-maytansinoid for 5 consecutive days. The tumors again regressed in response to the treatment.

Despite its effectiveness at shrinking tumors and suppressing tumor growth, HERCEPTIN®-DM1 does not kill normal human cells, indicating a selective activity. The effect of various concentrations of HERCEPTIN®-DM1 on hummman mammary epithelial cells, human hepatocytes and human small airway epithelial cells was investigated. At antibody concentrations of up to 10 μg/ml, the conjugate had no significant effect on cell number.

The pharmacokinetics of HERCEPTIN®-DM1 was evaluated in mice and cynomolgous monkeys. It was determined that the HERCEPTIN®-DM1 pharmacokinetics was linear with respect to dose in both mouse and cynomolgous monkeys following i.v. administration. Dose response analysis in mice indicated that tumor suppression increased with increasing exposure to HERCEPTIN®-DM1 and reached maximum suppression following a dose of at least 18 mg/kg given once a week. The concentration-effect relationship will be further characterized in future studies.

FIG. 11 shows the results of treating mice with HER-2 over-expressing tumors with HERCEPTIN®-DM1 once a week for 5 weeks. Each dose contained 300 μg/kg of DM1 and 18 mg/kg of HERCEPTIN®. Another group of animals received HERCEPTIN® twice a week at a dose of 18 mg/kg body weight. Animals that did not receive any treatment were used as control. As shown in FIG. 11, in animals that received HERCEPTIN®-DM1 tumor size was dramatically controlled and, perhaps more importantly, the tumor size was kept under control even after the therapy was stopped.

In yet another experiment, the amount of HERCEPTIN®-DM1 conjugate as well as the frequency of administration were varied while keeping the total number of doses at five. The evaluation was carried out in nude mice containing HER2 tumor transplants in mammary pads. As shown in FIG. 12, some animals received HERCEPTIN®-DM1 at 300, 100, 30 or 10 μg DM1/kg twice a week for the total of 5 doses. Another group of animals received HERCEPTIN®-DM1 at 300 or 100 μg DM1/kg once a week for the total of 5 doses. For comparison, HERCEPTIN® alone was administered at 18 mg/kg twice a week or a control monoclonal antibody (E25 directed against CD20, also known as RITUXAN®) was administered twice a week. Consistent with earlier results in this HERCEPTIN® insensitive model, HERCEPTIN® failed to control the growth of mammary tumors. However, HERCEPTIN®-DM1 conjugate showed

dramatic anti-tumor activity in a dose-dependent manner. For example, higher doses showed more potent anti-tumor activity than lower doses in both of the groups, i.e. those receiving treatments twice a week and once a week. Furthermore, twice a week treatment was more effective in keeping the tumor size smaller than once a week treatment schedule. Interestingly, however, there was no significant difference in the profile of tumor growth inhibition at 300 μg DM1/kg dose of HERCEPTIN®-DM1 conjugate whether administered twice a week or once a week. This suggests that at 300 μg DM1/kg dose, once a week schedule of treatment is effective in controlling tumor growth and that a higher frequency of administration is not necessary.

The results of a similar experiment are depicted in FIG. **13**. The results of three different dosing regimens of HERCEPTIN®-DM1 conjugate on tumor size are shown compared to matching dosing regimens of RITUXAN®-DM1. Tumor size was reduced and tumor growth was suppressed for at least about 50 days by treatment with 5 doses of HERCEPTIN®-DM1 at a concentration of 300 μg DM1/kg. This was true both when the HERCEPTIN®-DM1 was administered once a week and when it wsa administered twice a week. By contrast, administration of 5 doses of HERCEPTIN®-DM1 twice a week at a concentration of 100 μg DM1/kg did not shrink tumor size and suppressed tumor growth for somewhat less time. Matched RITUXAN®-DM1 treatment showed little effect on tumor size, indicating that the observed effect is specific to HERCEPTIN®-DM1. Similarly, unconjugated RITUXAN® (control MAb E25) showed no efficacy.

As can be seen clearly in FIG. **14**, a dose of HERCEPTIN®-DM1 (300 μg DM1/kg) once a week for five weeks caused tumors to shrink and prevented regrowth for more than 60 days. FIG. **14** also shows that a second round of treatment with HERCEPTIN®-DM1 after tumor regrowth begins is capable of shrinking tumors a second time. Neither unconjugated RITUXAN® (control MAb E25) nor RITUXAN®-DM1 had any obvious effect on tumor growth.

As shown here, the mammary tumor transplanted from HER2 transgenic mouse serves as a very useful model in evaluating various anti-tumor compounds as well as in establishing efficacy of different treatment regimens in preclinical studies. The model is particularly unique as it shares an important attribute of a fraction of human mammary tumors which are either completely or partially refractory to the treatment of HERCEPTIN® inspite of overexpression of HER2 at 2+ or 3+ levels. Thus, HER2 transgenic model described herein provides a valuable tool not only to study the mechanism of resistance to the anti-tumor activity of HERCEPTIN®, but also for screening compounds or modified HERCEPTIN®, including conjugates, for anti-tumor activity. The insight gained from such studies is likely to help in developing effective breast cancer therapies with broad coverage including HERCEPTIN®-resistant metastatic HER2 overexpressing breast carcinomas. The present HER2 transgenic model is particularly suited for preelinical research as well as drug development, and is a better alternative to in vitro studies carried out using breast carcinoma cell lines. It is an in vivo system with normal stroma and microenvironment of breast epithelium with cell-cell and cell-matrix interactions that are typical of a tissue. It also takes into account local factors and cytokines produced in the normal course of mammary gland development and attendant regulatory networks. It is also suitable to carry out pharmacokinetics studies of drug candidates at a smaller scale, which can then be scaled up for studies in non-human primate models. The results provide a sound basis for actual clinical trials in human subjects. As per the strategy described herein, the development of a HER2 transgenic model does not need to involve any in vitro selection, and requires minimal in vivo selection, the latter being limited to the extent of serial passage of the mammary tissue in order to reduce the duration of time needed to develop tumors and obtaining homogenous overexpression of HER2 in mammary cells. Moreover, breeding of these mice provides a continuous source of tissues for various follow-up or supplemental studies. This is particularly significant since the availability of clinical samples of tissues from breast cancer patients is highly limited.

The HERCEPTIN®-DM1 conjugate as described herein was found to have superior activity over HERCEPTIN® in this HER2 transgenic model that mimics HERCEPTIN®-resistant metastatic HER2 overexpressing breast carcinomas. Approximately, 85% of breast cancer patients either do not respond to HERCEPTIN therapy or repond poorly. The molecular basis of the resistance is not clearly understood. However, it is not due to a lower level of HER2 expression since these tumors also overexpress HER2 at 2+ or 3+ levels. Nevertheless, this significant proportion of breast cancer patients is not able to avail themselves of the powerful potential of HERECEPTIN® therapy. Preclinical studies carried out using the HERCEPTIN®-insensitive HER2 transgenic mouse model as outlined in this application shows a dramatic response of these tumors to HERCEPTIN®-DM1 conjugate as compared to HERCEPTIN®.

The HERCEPTIN®-DM1 conjugate was found to effectively control the growth of HERCEPTIN®-resistant tumors in a dose-dependent manner at a dosage of 100 μg DM1/kg and above. Administration of the tested conjugate at 300 μg DM1/kg once a week brought about a very impressive inhibition of tumor growth. Five such doses completely prevented the emergence of tumor for more than 60 days, and when the tumor did begin to reemerge, a second round of HERCEPTIN®-DM1 was able to control the growth. This is in contrast with a rapid growth of tumors in animals treated with control monoclonal antibody (RITUXAN®), maytansinoid conjugated control monoclonal antibody (RITUXAN®-DM1) or unconjugated HERCEPTIN®. Thus, the preclinical studies presented herein clearly show that the HERCEPTIN®-DM1 conjugate is able to elicit a dramatic anti-tumor response even in HERCEPTIN®-resistant breast tumors. The better objective response rate obtained with the HERCEPTIN®-DM1 conjugate will allow a higher fraction of breast cancer patients to benefit from this powerful therapy. The fact that the effect of HERCEPTIN®-DM1 is dose-dependent suggests that in an actual clinical setting, the strategy is likely to provide a considerable maneuver of doses to achieve the best anti-tumor activity. Moreover, the duration of anti-tumor response is significantly longer, permitting less frequent administration of the conjugate without compromising the therapeutic efficacy. The resultant cost-effectiveness and convenience is quite significant. Furthermore, the conjugate is likely to improve survival rate among the responders. For example, the median time to disease progression in HERCEPTIN® treated patients was only 3.1 months. With the superior therapeutic efficay of HERCEPTIN®-DM1 as compared to HERCEPTIN®, survival rate is likely to be increased.

All references cited throught the specification, and the references cited therein, are hereby expressly incorporated by reference.

Deposit of Biological Material

The following hybridoma cell lines have been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Va. 20110-2209, USA (ATCC):

Antibody Designation	ATCC No.	Deposit Date
7C2	ATCC HB-12215	October 17, 1996
7F3	ATCC HB-12216	October 17, 1996
4D5	ATCC CRL 10463	May 24, 1990
2C4	ATCC HB-12697	April 8, 1999

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of the deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.12 with particular reference to 886 OG 638).

In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)

The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the constructs deposited, since the deposited embodiments are intended to illustrate only certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that they represent. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

It is understood that the application of the teachings of the present invention to a specific problem or situation will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the products of the present invention and representative processes for their isolation, use, and manufacture appear below, but should not be construed to limit the invention.

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Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser Lys Asn Thr Leu Tyr
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
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Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65                  70                  75                  80

Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
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Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
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Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
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Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
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Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
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Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
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Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
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His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
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Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
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His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
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Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
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Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
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Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
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Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
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Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
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What is claimed is:

1. A method for the treatment of a tumor in a mammal, comprising the steps of (i) identifying said tumor as being characterized by overexpression of an ErbB2 receptor and as being a tumor that does not respond, or responds poorly, to treatment with an anti-ErbB2 antibody which binds to the 4D5 epitope and which has a growth inhibitory effect on SK-BR-3 cells, and (ii) administering to a mammal having said tumor a therapeutically effective amount of a conjugate of an anti-ErbB2 antibody which binds to the 4D5 epitope with a maytansinoid.

2. The method of claim 1 wherein the mammal is human.

3. The method of claim 1 wherein the anti-ErbB2 antibody is a growth inhibitory antibody effective to inhibit the growth of SK-BR-3 breast tumor cells in vitro.

4. The method of claim 1 wherein the anti-ErbB2 antibody induces cell death when applied at an effective concentration in vitro to SK-BR-3 cells.

5. The method of claim 1 wherein the anti-ErbB2 antibody induces apoptosis when applied at an effective concentration in vitro to SK-BR-3 cells.

6. The method of claim 1 wherein the tumor is cancer.

7. The method of claim 6 wherein the cancer is selected from the group consisting of breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer.

8. The method of claim 7 wherein the cancer is breast cancer.

9. The method of claim 8 wherein the breast cancer overexpresses ErbB2 at a 2+ level or more.

10. The method of claim 9 wherein the breast cancer overexpresses ErbB2 at a 3+ level.

11. The method of claim 10 wherein the breast cancer is a metastatic breast cancer.

12. The method of claim 10 wherein the antibody has a biological characteristic of a 4D5 monoclonal antibody (ATCC CRL 10463) such that the antibody shows a growth inhibitory effect on SK-BR-3 cells in a manner that is dependent on the ErbB2 expression level and/or blocks binding of monoclonal antibody 4D5 to ErbB2.

13. The method of claim 12 wherein the antibody binds essentially the same epitope as a 4D5 monoclonal antibody (ATCC CRL 10463).

14. The method of claim 12 wherein the antibody is the monoclonal antibody 4D5 (ATCC CRL 10463).

15. The method of claim 12 wherein the antibody is humanized.

16. The method of claim 15 wherein the antibody is selected from the group consisting of humanized antibodies huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8.

17. The method of claim 16 wherein the antibody is humanized antibody huMAb4D5-8.

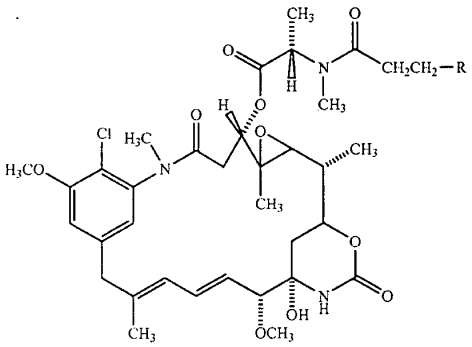
18. The method of claim 1 wherein the antibody is an antigen binding fragment of said antibody.

19. The method of claim 18 wherein the antibody binding is selected from the group a consisting of a Fab, Fab', $F(ab')_2$, F_v fragment, diabody, linear antibody, and single-chain antibody molecule.

20. The method of claim 1 wherein the maytansinoid is a maytansinol ester.

21. The method of claim 20 wherein the maytansinoid is a C-3 ester of maytansinol.

22. The method of claim 21 wherein the maytansinoid is DM1 having the structure



wherein R is SH.

23. The method of claim 1 wherein the antibody and maytansinoid are conjugated by a bispecific chemical linker.

24. The method of claim 23 wherein said chemical linker is N-succinimidyl-4(2-pyridylthio)propanoate (SPDP), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) or N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP).

25. The method of claim 1 wherein the antibody and maytansinoid are conjugated by a linking group selected from the group consisting of a disulfide, thioether, acid labile, photolabile, peptidase labile, and esterase labile group.

26. The method of claim 25 wherein the linking group is a disulfide or a thioether group.

27. The method of claim 26 wherein the linking group comprises a disulfide group.

28. The method of claim 1 wherein the conjugate comprises 1 to about 10 maytansinoid molecules per antibody molecule.

29. The method of claim 28 wherein the conjugate comprises from about 3 to about 5 maytansinoid molecules per antibody molecule.

30. The method of claim 1 further comprising the administration of a second antibody which binds ErbB2.

31. The method of claim 30 wherein the second antibody comprises monoclonal antibody 2c4 (ATCC HB-12697) or humanized 2C4.

32. The method of claim 30 wherein the second antibody is humanized antibody, huMAb4D5-8.

33. The method of claim 1 wherein treatment with the conjugate is followed by treatment with an unconjugated anti-ErbB antibody.

34. The method of claim 28 wherein the conjugate is administered weekly at a dose of 0.1 to 10 mg/kg body weight.

35. The method of claim 34 wherein said administration is followed by a dose of 0.3 mg/kg body weight approximately 10 weeks later.

36. The method of claim 29 wherein the conjugate is administered weekly at a dose of 1 to 3 mg/kg body weight.

37. The method of claim 36 wherein said administration is followed by a dose of 0.3 mg/kg body weight approximately 10 weeks later.

38. The method of claim 1 wherein the conjugate is administered weekly at a dose of 0.1 to 5 mg/kg body weight for 4 to 6 weeks, followed by maintenance treatment with unconjugated anti-ErbB2 antibody.

39. The method of claim 38 wherein the unconjugated antibody is humanized antibody huMAb4D5-8 or humanized 2C4.

40. The method of claim 30 wherein said second antibody is conjugated with a cytotoxic agent.

41. The method of claim 40 wherein the cytotoxic agent is a maytansinoid.

42. The method of claim 1 wherein said treatment has an improved objective response rate compared to treatment with huMAb4D5-8 alone.

43. The method of claim 1 wherein said treatment has a longer duration of response than treatment with huMAb4D5-8 alone.

44. The method of claim 1 wherein said treatment results in increased survival of the mammal treated compared with treatment with huMAb4D5-8 alone.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,097,840 B2
APPLICATION NO. : 09/811123
DATED : March 16, 2001
INVENTOR(S) : Sharon Erickson et al.

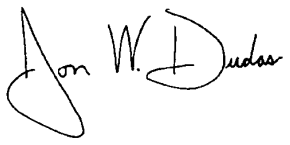
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Col. 84, lines 15-18, should read,

19. The method of claim 18 wherein the antigen binding fragment is selected from the group a consisting of a Fab, Fab', $F(ab')_2$, F_v fragment, diabody, linear antibody, and a single-chain antibody molecule.

Signed and Sealed this

Twelfth Day of December, 2006



JON W. DUDAS
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,097,840 B2
APPLICATION NO. : 09/811123
DATED : August 29, 2006
INVENTOR(S) : Sharon Erickson et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Col. 84, lines 15-18, should read,

19. The method of claim 18 wherein the antigen binding fragment is selected from the group a consisting of a Fab, Fab', $F(ab')_2$, F_v fragment, diabody, linear antibody, and a single-chain antibody molecule.

This certificate supersedes Certificate of Correction issued December 12, 2006.

Signed and Sealed this

Ninth Day of January, 2007

JON W. DUDAS
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,097,840 B2
APPLICATION NO. : 09/811123
DATED : August 29, 2006
INVENTOR(S) : Sharon Erickson et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Col. 84, lines 15-18, should read,

19. The method of claim 18 wherein the antigen binding fragment is selected from the group consisting of a Fab, Fab', $F(ab')_2$, F_v fragment, diabody, linear antibody, and single-chain antibody molecule.

This certificate supersedes Certificate of Correction issued December 12, 2006 and January 9, 2007.

Signed and Sealed this

Twentieth Day of March, 2007

JON W. DUDAS
Director of the United States Patent and Trademark Office

Attachment E

Certificate of Correction for U.S. Patent No. 7,097,840

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,097,840 B2
APPLICATION NO. : 09/811123
DATED : August 29, 2006
INVENTOR(S) : Sharon Erickson et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

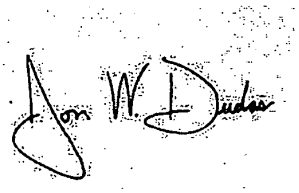
Col. 84, lines 15-18, should read,

19. The method of claim 18 wherein the antigen binding fragment is selected from the group consisting of a Fab, Fab', $F(ab')_2$, F_v fragment, diabody, linear antibody, and single-chain antibody molecule.

This certificate supersedes Certificate of Correction issued December 12, 2006 and January 9, 2007.

Signed and Sealed this

Twentieth Day of March, 2007



JON W. DUDAS
Director of the United States Patent and Trademark Office

Attachment F

Evidence of Maintenance Fee Schedule for U.S. Patent No. 7,097,840

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United States Patent and Trademark Office

Patent Bibliographic Data					03/25/2013 02:27 PM
Patent Number:	7097840		Application Number:	09811123	
Issue Date:	08/29/2006		Filing Date:	03/16/2001	
Title:	METHODS OF TREATMENT USING ANTI-ERBB ANTIBODY-MAYTANSINOID CONJUGATES				
Status:	8th year fee window opens: 08/29/2013			Entity:	LARGE
Window Opens:	N/A	Surcharge Date:	N/A	Expiration:	N/A
Fee Amt Due:	Window not open	Surchg Amt Due:	Window not open	Total Amt Due:	Window not open
Fee Code:					
Surcharge Fee Code:					
Most recent events (up to 7):	01/29/2010	Payment of Maintenance Fee, 4th Year, Large Entity. --- End of Maintenance History ---			
Address for fee purposes:	Goodwin Procter LLP Attn: Patent Administrator 135 Commonwealth Drive Menlo Park CA 94025-1105				

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Attachment G

Drug details for Herceptin®

U.S. Food & Drug Administration

Drugs@FDA
FDA Approved Drug Products

Drug Details

Drug Name(s)	**HERCEPTIN**
FDA Application No.	**(BLA) 103792**
Active Ingredient(s)	**TRASTUZUMAB**
Company	**GENENTECH**
Original Approval or Tentative Approval Date	**September 25, 1998**

- **There are no Therapeutic Equivalents**
- **Approval History, Letters, Reviews, and Related Documents**
- **Label Information**

Products on Application (BLA) #103792
Click on a column header to re-sort the table:

Drug Name	Active Ingredients	Strength	Dosage Form/Route	Marketing Status	RLD	TE Code
HERCEPTIN	TRASTUZUMAB	21MG/ML	VIAL; INTRAVENOUS	Prescription	No	None

Back to Top | Back to Previous Page | Back to Drugs@FDA Home

Disclaimer[10]

FDA/Center for Drug Evaluation and Research
Office of Communications
Division of Online Communications
Update Frequency: Daily

Links on this page:

1. http://www.addthis.com/bookmark.php?u508=true&v=152&username=fdamain
2. http://www.addthis.com/bookmark.php
3. http://www.fda.gov/default.htm
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5. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm
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Note: If you need help accessing information in different file formats, see Instructions for Downloading Viewers and Players.

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- Contact FDA
- Careers
- FDA Basics

- FOIA
- No Fear Act
- Site Map
- Transparency
- Website Policies

U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
Ph. 1-888-INFO-FDA (1-888-463-6332)
Email FDA

- USA.gov
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- Combination Products
- Advisory Committees
- Science & Research
- Regulatory Information
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- Emergency Preparedness
- International Programs
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- Training and Continuing Education
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- State & Local Officials
- Consumers
- Industry
- Health Professionals

 U.S. Department of **Health & Human Services**

Links on this page:

Attachment H

Letter from the FDA to Genentech, Inc. regarding receipt and acceptance of BLA Application

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

BLA 125427/0000

BLA ACKNOWLEDGEMENT

Genentech, Inc.
Attention: Erica J. Evans, Ph.D.
Regulatory Program Management
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Evans:

We have received your Biologics License Application (BLA) submitted under section 351(a)/351(k) of the Public Health Service Act (PHS Act) for the following:

Name of Biological Product: Kadcyla (trastuzumab emtansine)

Date of Application: August 24, 2012

Date of Receipt: August 27, 2012

Our Secondary Tracking Number (STN): BLA 125427/ 0000

Proposed Use: single agent for treatment of patients with HER2-positivie metastatic breast cancer

If you have not already done so, promptly submit the content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format as described at http://www.fda.gov/oc/datacouncil/spl.html. Failure to submit the content of labeling in SPL format may result in a refusal-to-file action. The content of labeling must conform to the format and content requirements of 21 CFR 201.56-57.

You are also responsible for complying with the applicable provisions of sections 402(i) and 402(j) of the Public Health Service Act (PHS Act) [42 USC §§ 282 (i) and (j)], which was amended by Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law No, 110-85, 121 Stat. 904).

The BLA Submission Tracking Number provided above should be cited at the top of the first page of all submissions to this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Products 1
5901-B Ammendale Road
Beltsville, MD 20705-1266

All regulatory documents submitted in paper should be three-hole punched on the left side of the page and bound. The left margin should be at least three-fourths of an inch to assure text is not obscured in the fastened area. Standard paper size (8-1/2 by 11 inches) should be used; however, it may occasionally be necessary to use individual pages larger than standard paper size. Non-standard, large pages should be folded and mounted to allow the page to be opened for review without disassembling the jacket and refolded without damage when the volume is shelved. Shipping unbound documents may result in the loss of portions of the submission or an unnecessary delay in processing which could have an adverse impact on the review of the submission.

Secure email between CDER and applicants is useful for informal communications when confidential information may be included in the message (for example, trade secrets or patient information). If you have not already established secure email with the FDA and would like to set it up, send an email request to SecureEmail@fda.hhs.gov. Please note that secure email may not be used for formal regulatory submissions to applications.

If you have any questions, call me, Lisa Skarupa at (301) 796-2219.

Sincerely,

{See appended electronic signature page}

Lisa Skarupa, R.N., M.S.N., A.O.C.N.
Regulatory Project Manager
Division of Oncology Products 1
Office of Hematology and Oncology Products
Center for Drug Evaluation and Research

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LISA M SKARUPA
10/23/2012

Attachment I

Power of Attorney from Genentech, Inc. to Practitioners

Docket No.: 146392019700
Client Ref. No.: 10813

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Sharon Erickson *et al.*

Patent No.: 7,097,840

Issued: August 29, 2006

Application No: 09/811,123

For: METHODS OF TREATMENT USING ANTI-ERBB ANTIBODY-MAYTANSINOID CONJUGATES – Application for § 156 Patent Term Extension

Attorney Docket No: 146392019700

Assignees: Genentech, Inc. and ImmunoGen, Inc.

Unit: Office of Patent Legal Administration

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AUTHORIZATION AND POWER OF ATTORNEY TO FILE APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

As an authorized representative of Genentech, I hereby authorize Genentech's counsel, Morrison & Foerster LLP, to file and prosecute a patent term extension application under 35 U.S.C. § 156 for U.S. Patent 7,097,840 (the "'840 Patent"). Genentech, Inc., and ImmunoGen, Inc., are co-owners of the entire right, title and interest in the '840 Patent, and Genentech is the exclusive licensee of ImmunoGen, Inc.'s interest in the '840 Patent. Genentech is authorized to act as the agent of ImmunoGen, Inc. with respect to submission of this patent term extension application.

Accordingly, Genentech appoints practitioners associated with Customer Number 25226 to file and prosecute the patent term extension application for the '840 Patent and to transact all business in the United States Patent and Trademark Office connected with this patent term

extension application. Please direct all correspondence regarding this application for patent term extension to Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, CA 94304-1018. The correspondence address for the '840 Patent is to be unchanged for all other purposes.

Date 4/15/2013

Respectfully submitted,

By _____

Name: Paul Naik, Ph.D., J.D.

Title: Vice President, Intellectual Property

Phone: (650) 225-5530

pa-1582244