IV. Abstracts (continued)

24. Mbidde EK, Kazura J, Banura C, Hom D, Hellmann SD, Kizito A, and Hellmann N. Changing pattern of childhood Kaposi's sarcoma. Abstract # 228. IV Conference on AIDS and Associated Cancers in Africa, Marseille, France, 18-20 Oct. 1989.

25. Louie L, Desmond S, Gilles K, Newman B, King MC. Genetic Epidemiology of AIDS. Abstract # ThAP 83, V Int. Conf. on AIDS. Montreal Canada, June 4-9, 1989. page 154.

26. Heyer DM, Desmond SD, Volberding P, Kahn J. Changing Prevalence of Malignancies in Men at San Francisco General Hospital During the HIV Epidemic. Abstract # WBO 19, V Int. Conference on AIDS. Montreal Canada, June 4-9, 1989. page 206.

27. Kahn J, Desmond S, Bottles K, Kaplan L. Incidence of Malignancies in Men at San Francisco General Hospital during the AIDS Epidemic. Abstract # 7613, IV Int. Conference on AIDS. Stockholm Sweden, June 13-16, 1988, page 328.

Table 14
Time (Months) to Disease Progression in Study H0648g: Kaplan-Meier Statistics
(by FISH Availability)
Chemotherapy Received on Day 1

		AC		Paclitaxel		Total	
Patient Group		Herceptin + AC (N=143)	AC Alone (N=138)	Herceptin + Paclitaxel (N=92)	Paclitaxel Alone (N=96)	Herceptin +Chemotherapy (N=235)	Chemotherapy Alone (N=234)
IHC 2+/3+	N	143	138	92	96	235	234
	No. of Events	97	119	63	90	160	209
	No. of Censored Obs.	46	19	29	6	75	25
	% Censored	32.2%	13.8%	31.5%	6.3%	31.9%	10.7%
	Median	7.8	6.1	6.9	2.8	7.4	4.6
	95% C.I.	(7.3,9.4)	(4.9,7.1)	(5.3,9.9)	(2.0,4.3)	(7.0,9.0)	(4.4,5.4)
	25-75 %ile	4.8 - 12.7	3.7 - 9.7	3.7 - 12.6	1.6 - 5.4	4.4 - 12.7	2.0 - 7.6
	Min - Max	0.26* - 23.91*	0.20* - 26.74*	0.00* - 21.38*	0.03 - 13.29*	0.00* - 23.91*	0.03 - 26.74*
	P-value		0.0002		0.0001		0.0001

Results are based on all enrolled patients.
Note: . Indicates that Kaplan-Meier estimates are not available due to extensive censoring.
* Indicates censored observation. p-values are computed using log-rank test.

Source: Biostatistics(aiyuwu) pgm(/immuno/her2/h0648g/label2000/biostat/t_pd_2trt_fish_avail)
Database Status: CLOSED (Clinical Data of 18OCT99, FISH Data of 11FEB00)
Label Amendment 2000: Generated 11JUL00 11:06 Page 1 of 1

EXHIBIT B

Table 1
Time (Months) to Disease Progression in Study H0650g: Kaplan-Meier Statistics

Patient Group		2 mg/kg (N=33)	4 mg/kg (N=25)	Total (N=58)
Received Prior Anthracycline	N	33	25	58
	No. of Events	28	21	49
	No. of Censored Obs.	5	4	9
	% Censored	15.2%	16.0%	15.5%
	Median	3.5	3.5	3.5
	95% C.I.	(3.3,6.3)	(1.9,5.6)	(2.8,5.5)
	25-75 %ile	1.9 - 12.4	1.8 - 7.1	1.8 - 8.7
	Min - Max	0.16 - 20.39*	0.72 - 13.82*	0.16 - 20.39*

Note: . Indicates that Kaplan-Meier estimates are not available due to extensive censoring.
* Indicates censored observation.

Source: Biostatistics(aiyuwu) pgm(/immuno/her2/h0650g/explore00/biostat/t_pd_prevanc)
Database Status: CLOSED (Clinical Data of 14OCT99, FISH Data of 11FEB00)
H0650g Exploratory Analysis 2000: Generated 08AUG00 15:07

EXHIBIT C

PDR® entry for
Taxol Injection (Bristol-Myers Squibb Oncology/Immunology)

Warnings

WARNING

TAXOL® (paclitaxel) Injection should be administered under the supervision of a physician experienced in the use of cancer chemotherapeutic agents. Appropriate management of complications is possible only when adequate diagnostic and treatment facilities are readily available.

Anaphylaxis and severe hypersensitivity reactions characterized by dyspnea and hypotension requiring treatment, angioedema, and generalized urticaria have occurred in 2%-4% of patients receiving TAXOL in clinical trials. Fatal reactions have occurred in patients despite premedication. All patients should be pretreated with corticosteroids, diphenhydramine, and H_2 antagonists. (See **DOSAGE AND ADMINISTRATION** section.) Patients who experience severe hypersensitivity reactions to TAXOL should not be rechallenged with the drug.

TAXOL therapy should not be given to patients with solid tumors who have baseline neutrophil counts of less than 1,500 cells/mm ^{3} and should not be given to patients with AIDS-related Kaposi's sarcoma if the baseline neutrophil count is less than 1000 cells/mm ^{3} . In order to monitor the occurrence of bone marrow suppression, primarily neutropenia, which may be severe and result in infection, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving TAXOL.

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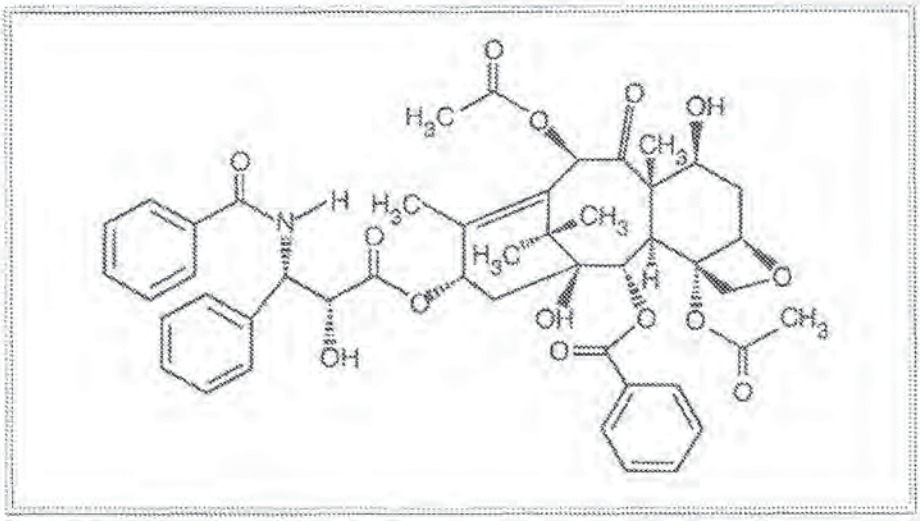
DESCRIPTION

TAXOL® (paclitaxel) Injection is a clear colorless to slightly yellow viscous solution. It is supplied as a nonaqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. TAXOL is available in 30 mg (5 mL), 100 mg (16.7 mL), and 300 mg (50 mL) multidose vials. Each mL of sterile nonpyrogenic solution contains 6 mg paclitaxel, 527 mg of purified Cremophor® EL * (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP.

Paclitaxel is a natural product with antitumor activity. TAXOL is obtained via a semi-synthetic process from *Taxus baccata* . The chemical name for paclitaxel is 5(beta),20-Epoxy-1,2(alpha),4,7(beta),10(beta),13(alpha)-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2 *R* ,3 *S*)- *N* -benzoyl-3-phenylisoserine.

Paclitaxel has the following structural formula:

EXHIBIT D



Paclitaxel is a white to off-white crystalline powder with the empirical formula $C_{47}H_{51}NO_{14}$ and a molecular weight of 853.9. It is highly lipophilic, insoluble in water, and melts at around 216-217°C.

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CLINICAL PHARMACOLOGY

Paclitaxel is a novel antimicrotubule agent that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. In addition, paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

Following intravenous administration of TAXOL, paclitaxel plasma concentrations declined in a biphasic manner. The initial rapid decline represents distribution to the peripheral compartment and elimination of the drug. The later phase is due, in part, to a relatively slow efflux of paclitaxel from the peripheral compartment.

Pharmacokinetic parameters of paclitaxel following 3- and 24-hour infusions of TAXOL at dose levels of 135 and 175 mg/m ^{2} were determined in a Phase 3 randomized study in ovarian cancer patients and are summarized in the following table:

Table 1: Summary of Pharmacokinetic Parameters - Mean Values

Dose (mg/m ^{2})	Infusion Duration (h)	N (patients)	C_{max} (ng/mL)	AUC (0-(infinity)) (ng·h/mL)	T-HALF (h)	CL_T (L/h/m ^{2})
135	24	2	195	6300	52.7	21.7
175	24	4	365	7993	15.7	23.8
135	3	7	2170	7952	13.1	17.7
175	3	5	3650	15007	20.2	12.2

C_{max} = Maximum plasma concentration

AUC (0-(infinity)) = Area under the plasma concentration-time curve from time 0 to infinity

CL_T = Total body clearance

It appeared that with the 24-hour infusion of TAXOL, a 30% increase in dose (135 mg/m ^{2} versus 175 mg/m ^{2}) increased the C_{max} by 87%, whereas the AUC (0-(infinity)) remained proportional. However, with a 3-hour infusion, for a 30% increase in dose, the C_{max} and AUC (0-(infinity)) were increased by 68% and 89%, respectively. The mean apparent volume of distribution at steady state, with the 24-hour infusion of TAXOL, ranged from 227 to 688 L/m ^{2} , indicating extensive extravascular distribution and/or tissue binding of paclitaxel.

The pharmacokinetics of paclitaxel were also evaluated in adult cancer patients who received single doses of 15-135 mg/m ^{2} given by 1-hour infusions (n=15), 30-275 mg/m ^{2} given by 6-hour infusions (n=36), and 200-275 mg/m ^{2} given by 24-hour infusions (n=54) in Phase 1 & 2 studies. Values for CL_T and volume of distribution were consistent with the findings in the Phase 3 study. The pharmacokinetics of TAXOL in patients with AIDS-related Kaposi's sarcoma have not been studied.

In vitro studies of binding to human serum proteins, using paclitaxel concentrations ranging from 0.1 to 50 µg/mL, indicate that between 89%-98% of drug is bound; the presence of cimetidine, ranitidine, dexamethasone, or diphenhydramine did not affect protein binding of paclitaxel.

After intravenous administration of 15-275 mg/m ^{2} doses of TAXOL as 1-, 6-, or 24-hour infusions, mean values for cumulative urinary recovery of unchanged drug ranged from 1.3% to 12.6% of the dose, indicating extensive non-renal clearance. In five patients administered a 225 or 250 mg/m ^{2} dose of radiolabeled TAXOL as a 3-hour infusion, a mean of 71% of the radioactivity was excreted in the feces in 120 hours, and 14% was recovered in the urine. Total recovery of radioactivity ranged from 56% to 101% of the dose. Paclitaxel represented a mean of 5% of the administered radioactivity recovered in the feces, while metabolites, primarily 6(alpha)-hydroxypaclitaxel, accounted for the balance. *In vitro* studies with human liver microsomes and tissue slices showed that paclitaxel was metabolized primarily to 6(alpha)-hydroxypaclitaxel by the cytochrome P450 isozyme CYP2C8; and to two minor metabolites, 3'- *p* -hydroxypaclitaxel and 6(alpha), 3'- *p* -dihydroxypaclitaxel, by CYP3A4. *In vitro* , the metabolism of paclitaxel to 6(alpha)-hydroxypaclitaxel was inhibited by a number of agents (ketoconazole, verapamil, diazepam, quinidine, dexamethasone, cyclosporin, teniposide, etoposide, and vincristine), but the concentrations used exceeded those found *in vivo* following normal therapeutic doses. Testosterone, 17(alpha)-ethinyl estradiol, retinoic acid, and quercetin, a specific inhibitor of CYP2C8, also inhibited the formation of 6(alpha)-hydroxypaclitaxel *in vitro* . The pharmacokinetics of paclitaxel may also be altered *in vivo* as a result of interactions with compounds that are substrates, inducers, or inhibitors of CYP2C8 and/or CYP3A4. (See **PRECAUTIONS: Drug Interactions** section.) The effect of renal or hepatic dysfunction on the disposition of paclitaxel has not been investigated.

Possible interactions of paclitaxel with concomitantly administered medications have not been formally investigated.

*Cremophor® EL is the registered trademark of BASF Aktiengesellshaft. Cremophor® EL is further purified by a Bristol-Myers Squibb Company proprietary process before use.
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CLINICAL STUDIES

Ovarian Carcinoma:

First-Line Data --The safety and efficacy of TAXOL (paclitaxel) Injection (135 mg/m ^{2} over 24 hours) in combination with cisplatin (75 mg/m ^{2}) in patients with advanced ovarian cancer and no prior chemotherapy were evaluated in a Phase 3 multicenter, randomized, controlled (vs. cyclophosphamide 750 mg/m ^{2} /cisplatin 75 mg/m ^{2}) clinical trial conducted by the Gynecologic Oncology Group (GOG). A total of 410 patients with Stage III or IV disease (>1 cm residual disease after staging laparotomy or

distant metastases) were randomized. Patients treated with TAXOL in combination with cisplatin had significantly longer time to progression (median 16.6 vs. 13.0 months, p=0.0008) and nearly a year longer median survival time (p=0.0002) compared with standard therapy.

Table 2: Efficacy in the Phase 3 First-Line Ovarian Carcinoma Study

	TAXOL/Cisplatin (n=196)		Cyclophosphamide/Cisplatin (n=214)
Clinical Response [a]	(n=113)		(n=127)
	62		48
		0.04	
Pathological Response [b]			
- rate (percent)	34		20
		0.001	
Pathological Complete Response			
- rate (percent)	21		16
		0.20	
Time to Progression			
- median (months)	16.6		13.0
- p-value		0.0008	
Survival			
- median (months)	35.5		24.2
- p-value		0.0002	

[a] Among evaluable patients only.

[b] Includes patients with pathological complete response plus patients with microscopic residual disease.

The adverse event profile for patients receiving TAXOL in combination with cisplatin in this study was generally consistent with that seen for the pooled analysis of data from 812 patients treated with single-agent TAXOL in 10 clinical studies. These adverse events and adverse events from the Phase 3 first-line ovarian carcinoma study are described in the **ADVERSE REACTIONS** section in tabular (Tables 8 and 9) and narrative form.

Second-Line Data --Data from five Phase 1 & 2 clinical studies (189 patients), a multicenter randomized Phase 3 study (407 patients), as well as an interim analysis of data from more than 300 patients enrolled in a treatment referral center program were used in support of the use of TAXOL in patients who have failed initial or subsequent chemotherapy for metastatic carcinoma of the ovary. Two of the Phase 2 studies (92 patients) utilized an initial dose of 135 to 170 mg/m ^{2} in most patients (>90%) administered over 24 hours by continuous infusion. Response rates in these two studies were 22% (95% CI: 11% to 37%) and 30% (95% CI: 18% to 46%) with a total of 6 complete and 18 partial responses in 92 patients. The median duration of overall response in these two studies measured from the first day of treatment was 7.2 months (range: 3.5-15.8 months) and 7.5 months (range: 5.3-17.4 months), respectively. The median survival was 8.1 months (range: 0.2-36.7 months) and 15.9 months (range: 1.8-34.5+ months).

The Phase 3 study had a bifactorial design and compared the efficacy and safety of TAXOL, administered at two different doses (135 or 175 mg/m ^{2}) and schedules (3- or 24-hour infusion). The overall response rate for the 407 patients was 16.2% (95% CI: 12.8% to 20.2%) with 6 complete and 60

partial responses. Duration of response, measured from the first day of treatment was 8.3 months (range: 3.2-21.6 months). Median time to progression was 3.7 months (range 0.1+ - 25.1+ months). Median survival was 11.5 months (range: 0.2-26.3+ months).

Response rates, median survival and median time to progression for the 4 arms are given in the following table.

Table 3: Efficacy in the Phase 3 Second-Line Ovarian Carcinoma Study

	175/3 (n=96)	175/24 (n=106)	135/3 (n=99)	135/24 (n=106)
· Response				
- rate (percent)	14.6	21.7	15.2	13.2
- 95% Confidence Interval	(8.5-23.6)	(14.5-31.0)	(9.0-24.1)	(7.7-21.5)
· Time to Progression				
- median (months)	4.4	4.2	3.4	2.8
- 95% Confidence Interval	(3.0-5.6)	(3.5-5.1)	(2.8-4.2)	(1.9-4.0)
· Survival				
- median (months)	11.5	11.8	13.1	10.7
- 95% Confidence Interval	(8.4-14.4)	(8.9-14.6)	(9.1-14.6)	(8.1-13.6)

Analyses were performed as planned by the bifactorial study design described in the protocol, by comparing the two doses (135 or 175 mg/m ^{2}) irrespective of the schedule (3 or 24 hours) and the two schedules irrespective of dose. Patients receiving the 175 mg/m ^{2} dose had a response rate similar to that for those receiving the 135 mg/m ^{2} dose: 18% vs. 14% (p=0.28). No difference in response rate was detected when comparing the 3-hour with the 24-hour infusion: 15% vs. 17% (p=0.50). Patients receiving the 175 mg/m ^{2} of TAXOL had a longer time to progression than those receiving the 135 mg/m ^{2} dose: median 4.2 vs. 3.1 months (p=0.03). The median time to progression for patients receiving the 3-hour vs. the 24-hour infusion was 4.0 months vs. 3.7 months, respectively. Median survival was 11.6 months in patients receiving the 175 mg/m ^{2} dose of TAXOL and 11.0 months in patients receiving the 135 mg/m ^{2} dose (p=0.92). Median survival was 11.7 months for patients receiving the 3-hour infusion of TAXOL and 11.2 months for patients receiving the 24-hour infusion (p=0.91). These statistical analyses should be viewed with caution because of the multiple comparisons made.

TAXOL remained active in patients who had developed resistance to platinum-containing therapy (defined as tumor progression while on, or tumor relapse within 6 months from completion of, a platinum-containing regimen) with response rates of 14% in the Phase 3 study and 31% in the Phase & 2 clinical studies.

The adverse event profile in this Phase 3 study was consistent with that seen for the pooled analysis of data from 812 patients treated in 10 clinical studies. These adverse events and adverse events from the Phase 3 second-line ovarian carcinoma study are described in the **ADVERSE REACTIONS** section in tabular (Tables 8 and 10) and narrative form.

The results of the randomized study support the use of TAXOL (paclitaxel) Injection at doses of 135 to 175 mg/m ^{2} , administered by a 3-hour intravenous infusion. The same doses administered by 24-hour infusion were more toxic. However, the study had insufficient power to determine whether a particular dose and schedule produced superior efficacy.

Breast Carcinoma: Data from 83 patients accrued in three Phase 2 open label studies and from 471 patients enrolled in a Phase 3 randomized study were available to support the use of TAXOL in patients

with metastatic breast carcinoma.

Phase 2 open label studies --Two studies were conducted in 53 patients previously treated with a maximum of one prior chemotherapeutic regimen. TAXOL was administered in these two trials as a 24-hour infusion at initial doses of 250 mg/m ^{2} (with G-CSF support) or 200 mg/m ^{2} . The response rates were 57% (95% CI: 37% to 75%) and 52% (95% CI: 32% to 72%), respectively. The third Phase 2 study was conducted in extensively pretreated patients who had failed anthracycline therapy and who had received a minimum of two chemotherapy regimens for the treatment of metastatic disease. The dose of TAXOL was 200 mg/m ^{2} as a 24-hour infusion with G-CSF support. Nine of the 30 patients achieved a partial response, for a response rate of 30% (95% CI: 15%-50%).

Phase 3 randomized study --This multicenter trial was conducted in patients previously treated with one or two regimens of chemotherapy. Patients were randomized to receive TAXOL at a dose of either 175 mg/m ^{2} or 135 mg/m ^{2} given as a 3-hour infusion. In the 471 patients enrolled, 60% had symptomatic disease with impaired performance status at study entry, and 73% had visceral metastases. These patients had failed prior chemotherapy either in the adjuvant setting (30%), the metastatic setting (39%), or both (31%). Sixty-seven percent of the patients had been previously exposed to anthracyclines and 23% of them had disease considered resistant to this class of agents.

The overall response rate for the 454 evaluable patients was 26% (95% CI: 22%-30%), with 17 complete and 99 partial responses. The median duration of response, measured from the first day of treatment, was 8.1 months (range: 3.4-18.1+ months). Overall for the 471 patients, the median time to progression was 3.5 months (range: 0.03-17.1 months). Median survival was 11.7 months (range: 0-18.9 months).

Response rates, median survival and median time to progression for the 2 arms are given in the following table.

Table 4: Efficacy in Breast Cancer after Failure of Initial Chemotherapy or Within 6 Months of Adjuvant Therapy

	175/3 (n=235)		135/3 (n=236)
Response			
- rate (percent)	28		22
- p-value		0.135	
Time to Progression			
- median (months)	4.2		3.0
- p-value		0.027	
Survival			
- median (months)	11.7		10.5
- p-value		0.321	

The adverse event profile of the patients who received single-agent TAXOL in the Phase 3 study was consistent with that seen for the pooled analysis of data from 812 patients treated in 10 clinical studies. These adverse events and adverse events from the Phase 3 breast carcinoma study are described in the **ADVERSE REACTIONS** section in tabular (Tables 8 and 11) and narrative form.

Non-Small Cell Lung Carcinoma (NSCLC) --In a Phase 3 open label randomized study conducted by the Eastern Cooperative Oncology Group (ECOG), 599 patients were randomized to either TAXOL (T) 135 mg/m ^{2} as a 24-hour infusion in combination with cisplatin (c) 75 mg/m ^{2}, TAXOL (T) 250 mg/m ^{2} as a 24-hour infusion in combination with cisplatin (c) 75 mg/m ^{2} with G-CSF support, or cisplatin (c) 75 mg/m ^{2} on day 1, followed by etoposide (VP) 100 mg/m ^{2} on days 1, 2, and 3 (control).

Response rates, median time to progression, median survival, and one-year survival rates are given in the following table. The reported p-values have not been adjusted for multiple comparisons. There were statistically significant differences favoring each of the TAXOL plus cisplatin arms for response rate and time to tumor progression. There was no statistically significant difference in survival between either TAXOL plus cisplatin arm and the cisplatin plus etoposide arm.

Table 5: Efficacy Parameters in the Phase 3 First-Line NSCLC Study

	T135/24 c75 (n=198)	T250/24 c75 (n=201)	VP100 [a] c75 (n=200)
Response			
- rate (percent)	25	23	12
- p-value [b]	0.001	<0.001	
Time to Progression			
- median (months)	4.3	4.9	2.7
- p-value [b]	0.05	0.004	
Survival			
- median (months)	9.3	10.0	7.4
- p-value [b]	0.12	0.08	
One-Year Survival			
- percent of patients	36	40	32

[a] Etoposide (VP) 100mg/m ^{2} was administered I.V. on days 1, 2 and 3.

[b] Compared to cisplatin/etoposide.

In the ECOG study, the Functional Assessment of Cancer Therapy-Lung (FACT-L) questionnaire had seven subscales that measured subjective assessment of treatment. Of the seven, the Lung Cancer Specific Symptoms subscale favored the TAXOL 135 mg/m ^{2} /24 hour plus cisplatin arm compared to the cisplatin/etoposide arm. For all other factors, there was no difference in the treatment groups.

The adverse event profile for patients who received TAXOL in combination with cisplatin in this study was generally consistent with that seen for the pooled analysis of data from 812 patients treated with single-agent TAXOL in 10 clinical studies. These adverse events and adverse events from the Phase 3 first-line NSCLC study are described in the **ADVERSE REACTIONS** section in tabular (Tables 8 and 12) and narrative form.

AIDS-Related Kaposi's Sarcoma: Data from two Phase 2 open label studies support the use of TAXOL as second-line therapy in patients with AIDS-related Kaposi's sarcoma. Fifty-nine of the 85 patients enrolled in these studies had previously received systemic therapy, including interferon alpha (32%), DaunoXome® (31%), DOXIL® (2%) and doxorubicin containing chemotherapy (42%), with 64% having received prior anthracyclines. Eighty-five percent of the pretreated patients had progressed on, or could not tolerate, prior systemic therapy.

In Study CA139-174 patients received TAXOL at 135 mg/m ^{2} as a 3-hour infusion every 3 weeks (intended dose intensity 45 mg/m ^{2} /week). If no dose-limiting toxicity was observed, patients were to receive 155 mg/m ^{2} and 175 mg/m ^{2} in subsequent courses. Hematopoietic growth factors were not to be used initially. In Study CA139-281 patients received TAXOL (paclitaxel) Injection at 100 mg/m ^{2} as a 3-hour infusion every 2 weeks (intended dose intensity 50 mg/m ^{2} /week). In this study patients could be receiving hematopoietic growth factors before the start of TAXOL therapy, or this support was to be initiated as indicated; the dose of TAXOL was not increased. The dose intensity of TAXOL used in this patient population was lower than the dose intensity recommended for other solid tumors.

DuanoXome® is a registered trademark of NeXstar Pharmaceuticals, Inc. DOXIL® is a registered trademark of Sequus Pharmaceuticals, Inc.

All patients had widespread and poor risk disease. Applying the ACTG staging criteria to patients with prior systemic therapy, 93% were poor risk for extent of disease (T_1), 88% had a CD4 count <200 cells/mm ^{3} (I_1), and 97% had poor risk considering their systemic illness (S_1).

All patients in Study CA139-174 had a Karnofsky performance status of 80 or 90 at baseline; in Study CA139-281, there were 26 (46%) patients with a Karnofsky performance status of 70 or worse at baseline.

Table 6: Extent of Disease at Study Entry

	Percent of Patients Prior Systemic Therapy (n=59)
Visceral ± edema ± oral ± cutaneous	42
Edema or lymph nodes oral ± cutaneous	41
Oral ± cutaneous	10
Cutaneous only	7

Although the planned dose intensity in the two studies was slightly different (45 mg/m ^{2} /week in Study CA139-174 and 50 mg/m ^{2} /week in Study CA139-281), delivered dose intensity was 38-39 mg/m ^{2} /week in both studies, with a similar range (20-24 to 51-61).

Efficacy— The efficacy of TAXOL was evaluated by assessing cutaneous tumor response according to the amended ACTG criteria and by seeking evidence of clinical benefit in patients in six domains of symptoms and/or conditions that are commonly related to AIDS-related Kaposi's sarcoma.

Cutaneous Tumor Response (Amended ACTG Criteria)— The objective response rate was 59% (95% CI: 46% to 72%) (35 of 59 patients) in patients with prior systemic therapy. Cutaneous responses were primarily defined as flattening of more than 50% of previously raised lesions.

Table 7: Overall Best Response (Amended ACTG Criteria)

	Percent of Patients Prior Systemic Therapy (n=59)
Complete response	3
Partial response	56
Stable disease	29
Progression	8
Early death/toxicity	3

The median time to response was 8.1 weeks and the median duration of response measured from the first day of treatment was 10.4 months (95% CI: 7.0 to 11.0 months) for the patients who had previously received systemic therapy. The median time to progression was 6.2 months (95% CI: 4.6 to 8.7 months).

Additional Clinical Benefit– Most data on patient benefit were assessed retrospectively (plans for such analyses were not included in the study protocols). Nonetheless, clinical descriptions and photographs indicated clear benefit in some patients, including instances of improved pulmonary function in patients with pulmonary involvement, improved ambulation, resolution of ulcers, and decreased analgesic requirements in patients with KS involving the feet and resolution of facial lesions and edema in patients with KS involving the face, extremities and genitalia.

Safety– The adverse event profile of TAXOL administered to patients with advanced HIV disease and poor-risk AIDS-related Kaposi's sarcoma was generally similar to that seen in a pooled analysis of data from 812 patients with solid tumors. These adverse events and adverse events from the Phase 2 second-line Kaposi's sarcoma studies are described in the **ADVERSE REACTIONS** section in tabular (Tables 8 and 13) and narrative form. In this immunosuppressed patient population, however, a lower dose intensity of TAXOL and supportive therapy including hematopoietic growth factors in patients with severe neutropenia are recommended. Patients with AIDS-related Kaposi's sarcoma may have more severe hematologic toxicities than patients with solid tumors.

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INDICATIONS

TAXOL is indicated as first-line and subsequent therapy for the treatment of advanced carcinoma of the ovary. As first-line therapy, TAXOL is indicated in combination with cisplatin.

TAXOL is indicated for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. Prior therapy should have included an anthracycline unless clinically contraindicated.

TAXOL, in combination with cisplatin, is indicated for the first-line treatment of non-small cell lung cancer in patients who are not candidates for potentially curative surgery and/or radiation therapy.

TAXOL is indicated for the second-line treatment of AIDS-related Kaposi's sarcoma.

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CONTRAINDICATIONS

TAXOL is contraindicated in patients who have a history of hypersensitivity reactions to TAXOL or other drugs formulated in Cremophor® EL (polyoxyethylated castor oil).

TAXOL should not be used in patients with solid tumors who have baseline neutrophil counts of <1500 cells/mm ^{3} or in patients with AIDS-related Kaposi's sarcoma with baseline neutrophil counts of <1000 cells/mm ^{3} .

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WARNINGS

Anaphylaxis and severe hypersensitivity reactions characterized by dyspnea and hypotension requiring treatment, angioedema, and generalized urticaria have occurred in 2%-4% of patients receiving TAXOL in clinical trials. Fatal reactions have occurred in patients despite premedication. All patients should be pretreated with corticosteroids, diphenhydramine, and H2 antagonists. (See **DOSAGE AND ADMINISTRATION** section.) Patients who experience severe hypersensitivity reactions to TAXOL should not be rechallenged with the drug.

Bone marrow suppression (primarily neutropenia) is dose-dependent and is the dose-limiting toxicity. Neutrophil nadirs occurred at a median of 11 days. TAXOL should not be administered to patients with baseline neutrophil counts of less than 1500 cells/mm ^{3} (<1000 cells/mm ^{3} for patients with KS). Frequent monitoring of blood counts should be instituted during TAXOL treatment. Patients should not be re-treated with subsequent cycles of TAXOL until neutrophils recover to a level >1500 cells/mm ^{3} (>1000 cells/mm ^{3} for patients with KS) and platelets recover to a level >100,000 cells/mm ^{3} .

Severe conduction abnormalities have been documented in <1% of patients during TAXOL therapy and in some cases requiring pacemaker placement. If patients develop significant conduction abnormalities during TAXOL infusion, appropriate therapy should be administered and continuous cardiac monitoring should be performed during subsequent therapy with TAXOL.

Pregnancy: TAXOL can cause fetal harm when administered to a pregnant woman. Administration of paclitaxel during the period of organogenesis to rabbits at doses of 3.0 mg/kg/day (about 0.2 the daily maximum recommended human dose on a mg/m ^{2} basis) caused embryo- and fetotoxicity, as indicated by intrauterine mortality, increased resorptions and increased fetal deaths. Maternal toxicity was also observed at this dose. No teratogenic effects were observed at 1.0 mg/kg/day (about 1/15 the daily maximum recommended human dose on a mg/m ^{2} basis); teratogenic potential could not be assessed at higher doses due to extensive fetal mortality.

There are no adequate and well-controlled studies in pregnant women. If TAXOL (paclitaxel) Injection is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

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PRECAUTIONS

Contact of the undiluted concentrate with plasticized polyvinyl chloride (PVC) equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP [di-(2-ethylhexyl)phthalate], which may be leached from PVC infusion bags or sets, diluted TAXOL solutions should preferably be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

TAXOL should be administered through an in-line filter with a microporous membrane not greater than 0.22 microns. Use of filter devices such as IVEX-2® filters which incorporate short inlet and outlet PVC-coated tubing has not resulted in significant leaching of DEHP.

Drug Interactions: In a Phase I trial using escalating doses of TAXOL (110-200 mg/m ^{2}) and cisplatin

(50 or 75 mg/m ^{2}) given as sequential infusions, myelosuppression was more profound when TAXOL was given after cisplatin than with the alternate sequence (i.e., TAXOL before cisplatin). Pharmacokinetic data from these patients demonstrated a decrease in paclitaxel clearance of approximately 33% when TAXOL was administered following cisplatin.

The metabolism of TAXOL is catalyzed by cytochrome P450 isoenzymes CYP2C8 and CYP3A4. In the absence of formal clinical drug interaction studies, caution should be exercised when administering TAXOL concomitantly with known substrates or inhibitors of the cytochrome P450 isoenzymes CYP2C8 and CYP3A4. (See **CLINICAL PHARMACOLOGY** section.)

Potential interactions between paclitaxel, a substrate of CYP3A4 and protease inhibitors (ritonavir, saquinavir, indinavir, and nelfinavir), which are substrates and/or inhibitors of CYP3A4 have not been evaluated in clinical trials.

Reports in the literature suggest that plasma levels of doxorubicin (and its active metabolite doxorubicinol) may be increased when paclitaxel and doxorubicin are used in combination.

Hematology: TAXOL therapy should not be administered to patients with baseline neutrophil counts of less than 1,500 cells/mm ^{3} . In order to monitor the occurrence of myelotoxicity, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving TAXOL. Patients should not be re-treated with subsequent cycles of TAXOL until neutrophils recover to a level >1500 cells/mm ^{3} and platelets recover to a level >100,000 cells/mm ^{3} . In the case of severe neutropenia (<500 cells/mm ^{3} for seven days or more) during a course of TAXOL therapy, a 20% reduction in dose for subsequent courses of therapy is recommended.

For patients with advanced HIV disease and poor-risk AIDS-related Kaposi's sarcoma, TAXOL, at the recommended dose for this disease, can be initiated and repeated if the neutrophil count is at least 1000 cells/mm ^{3} .

Hypersensitivity Reactions: Patients with a history of severe hypersensitivity reactions to products containing Cremophor® EL (e.g., cyclosporin for injection concentrate and teniposide for injection concentrate) should not be treated with TAXOL. In order to avoid the occurrence of severe hypersensitivity reactions, all patients treated with TAXOL should be premedicated with corticosteroids (such as dexamethasone), diphenhydramine and H_2 antagonists (such as cimetidine or ranitidine). Minor symptoms such as flushing, skin reactions, dyspnea, hypotension or tachycardia do not require interruption of therapy. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema or generalized urticaria require immediate discontinuation of TAXOL and aggressive symptomatic therapy. Patients who have developed severe hypersensitivity reactions should not be rechallenged with TAXOL.

Cardiovascular: Hypotension, bradycardia, and hypertension have been observed during administration of TAXOL, but generally do not require treatment. Occasionally TAXOL infusions must be interrupted or discontinued because of initial or recurrent hypertension. Frequent vital sign monitoring, particularly during the first hour of TAXOL infusion, is recommended. Continuous cardiac monitoring is not required except for patients with serious conduction abnormalities. (See **WARNINGS** section.)

Nervous System: Although, the occurrence of peripheral neuropathy is frequent, the development of severe symptomatology is unusual and requires a dose reduction of 20% for all subsequent courses of TAXOL.

TAXOL contains dehydrated alcohol USP, 396 mg/mL; consideration should be given to possible CNS and other effects of alcohol. (See **PRECAUTIONS: Pediatric Use** section.)

Hepatic: There is evidence that the toxicity of TAXOL is enhanced in patients with elevated liver enzymes. Caution should be exercised when administering TAXOL to patients with moderate to severe

hepatic impairment and dose adjustments should be considered.

Injection Site Reaction: Injection site reactions, including reactions secondary to extravasation, were usually mild and consisted of erythema, tenderness, skin discoloration, or swelling at the injection site. These reactions have been observed more frequently with the 24-hour infusion than with the 3-hour infusion. Recurrence of skin reactions at a site of previous extravasation following administration of TAXOL at a different site, i.e., "recall", has been reported rarely.

Rare reports of more severe events such as phlebitis, cellulitis, induration, skin exfoliation, necrosis and fibrosis have been received as part of the continuing surveillance of TAXOL safety. In some cases the onset of the injection site reaction either occurred during a prolonged infusion or was delayed by a week to ten days.

A specific treatment for extravasation reactions is unknown at this time. Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration.

Carcinogenesis, Mutagenesis, Impairment of Fertility: The carcinogenic potential of TAXOL has not been studied.

Paclitaxel has been shown to be clastogenic *in vitro* (chromosome aberrations in human lymphocytes) and *in vivo* (micronucleus test in mice). Paclitaxel was not mutagenic in the Ames test or the CHO/HGPRT gene mutation assay.

Administration of paclitaxel prior to and during mating produced impairment of fertility in male and female rats at doses equal to or greater than 1 mg/kg/day (about 0.04 the daily maximum recommended human dose on a mg/m ^{2} basis). At this dose, paclitaxel caused reduced fertility and reproductive indices, and increased embryo- and fetotoxicity. (See **WARNINGS** section.)

Pregnancy: Pregnancy "Category D". (See **WARNINGS** section.)

Nursing Mothers: It is not known whether the drug is excreted in human milk. Following intravenous administration of carbon-14 labeled TAXOL to rats on days 9 to 10 postpartum, concentrations of radioactivity in milk were higher than in plasma and declined in parallel with the plasma concentrations. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, it is recommended that nursing be discontinued when receiving TAXOL therapy.

Pediatric Use: The safety and effectiveness of TAXOL in pediatric patients have not been established.

There have been reports of central nervous system (CNS) toxicity (rarely associated with death) in a clinical trial in pediatric patients in which TAXOL was infused intravenously over 3 hours at doses ranging from 350 mg/m ^{2} to 420 mg/m ^{2}. The toxicity is most likely attributable to the high dose of the ethanol component of the TAXOL vehicle given over a short infusion time. The use of concomitant antihistamines may intensify this effect. Although a direct effect of the paclitaxel itself cannot be discounted, the high doses used in this study (over twice the recommended adult dosage) must be considered in assessing the safety of TAXOL for use in this population.

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ADVERSE REACTIONS

Pooled Analysis of Adverse Event Experiences from Single-Agent Studies: Data in the following table are based on the experience of 812 patients (493 with ovarian carcinoma and 319 with breast carcinoma) enrolled in 10 studies who received single-agent TAXOL (paclitaxel) Injection. Two hundred and seventy-five patients were treated in eight Phase 2 studies with TAXOL doses ranging from

135 to 300 mg/m ^{2} administered over 24 hours (in four of these studies, G-CSF was administered as hematopoietic support). Three hundred and one patients were treated in the randomized Phase 3 ovarian carcinoma study which compared two doses (135 or 175 mg/m ^{2}) and two schedules (3 or 24 hours) of TAXOL. Two hundred and thirty-six patients with breast carcinoma received TAXOL (135 or 175 mg/m ^{2}) administered over 3 hours in a controlled study.

Table 8: Summary [a] of Adverse Events in Patients With Solid Tumors Receiving Single-Agent TAXOL

		Percent of Patients (n=812)
· Bone Marrow		
- Neutropenia	< 2,000/mm ^{3} < 500/mm ^{3}	90 52
- Leukopenia	< 4,000/mm ^{3} < 1,000/mm ^{3}	90 17
- Thrombocytopenia	< 100,000/mm ^{3} < 50,000/mm ^{3}	20 7
- Anemia	< 11 g/dL < 8 g/dL	78 16
- Infections		30
- Bleeding		14
- Red Cell Transfusions		25
- Platelet Transfusions		2
· Hypersensitivity Reaction [b]		
- All		41
- Severe **		2
· Cardiovascular		
- Vital Sign Changes [c]		
- Bradycardia (N=537)		3
- Hypotension (N=532)		12
- Significant Cardiovascular Events		1
· Abnormal ECG		
- All Pts		23
- Pts with normal baseline (N=559)		14
· Peripheral Neuropathy		
- Any symptoms		60
- Severe symptoms **		3
· Myalgia/Arthralgia		
- Any symptoms		60
- Severe symptoms **		8
· Gastrointestinal		
- Nausea and vomiting		52
- Diarrhea		38

- Mucositis		31
· Alopecia		87
· Hepatic (Pts with normal baseline and on study data)		
- Bilirubin elevations (N=765)		7
- Alkaline phosphatase elevations (N=575)		22
- AST (SGOT) elevations (N=591)		19
· Injection Site Reaction		13
[a] Based on worst course analysis.		
[b] All patients received predmedication.		
[c] During the first 3 hours of infusion.		
** Severe events are defined as at least Grade III toxicity.		

None of the observed toxicities were clearly influenced by age.

Disease-Specific Adverse Event Experiences First-Line Ovary in Combination: For the 409 patients who were evaluable for safety in the Phase 3 first-line ovary combination therapy study, the following table shows the incidence of important adverse events.

Table 9: Frequency [a] of Important Adverse Events in the Phase 3 First-Line Ovarian Carcinoma Study

		Percent of Patients	
		TAXOL (135/24) [b] / Cisplatin (75) [c] (n=196)	Cyclophosphamide (750) [c] / Cisplatin (75) [c] (n=213)
· Bone Marrow			
- Neutropenia	< 2,000/mm ^{3} < 500/mm ^{3}	96 81 [d]	92 58 [d]
- Thrombocytopenia	< 100,000/mm ^{3} < 50,000/mm ^{3}	26 10	30 9
- Anemia	< 11 g/dL < 8 g/dL	88 13	86 9
- Infections		21	15
- Febrile Neutropenia		15 [d]	4 [d]
· Hypersensitivity Reaction [e]			
- All		8 [d]	1 [d]
- Severe **		3 [d]	-- [d]
· Peripheral Neuropathy			
- Any symptoms		25	20
- Severe symptoms **		3 [d]	-- [d]
· Nausea and Vomiting			
- Any symptoms		65	69

- Severe symptoms **		10	11
· Myalgia/Arthralgia			
- Any symptoms		9 [d]	2 [d]
- Severe symptoms **		1	–
· Diarrhea			
- Any symptoms		16 [d]	8 [d]
- Severe symptoms **		4	1
· Asthenia			
- Any symptoms		17 [d]	10 [d]
- Severe symptoms **		1	1
· Alopecia			
- Any symptoms		55 [d]	37 [d]
- Severe symptoms **		6	8

[a] Based on worst course analysis.

[b] TAXOL dose in mg/m ^{2} /infusion duration in hours.

[c] Dose in mg/m ^{2} .

[d] $p<0.05$ by Fisher exact test.

[e] All patients received premedication.

** Severe events are defined as at least Grade III toxicity.

Second-Line Ovary: For the 403 patients who received single-agent TAXOL (paclitaxel) Injection in the Phase 3 second-line ovarian carcinoma study, the following table shows the incidence of important adverse events.

Table 10: Frequency [a] of Important Adverse Events in the Phase 3 Second-Line Ovarian Carcinoma Study

		Percent of Patients			
		175/3 [b] (n=95)	175/24 [b] (n=105)	135/3 [b] (n=98)	135/24 [b] (n=105)
· Bone Marrow					
- Neutropenia	< 2,000/mm ^{3}	78	98	78	98
	< 500/mm ^{3}	27	75	14	67
- Thrombocytopenia	< 100,000/mm ^{3}	4	18	8	6
	< 50,000/mm ^{3}	1	7	2	1
	< 11 g/dL	84	90	68	88
	< 8 g/dL	11	12	6	10
		26	29	20	18
· Hypersensitivity Reaction [c]					
- All		41	45	38	45
- Severe **		2	0	2	1
· Peripheral Neuropathy					
- Any symptoms		63	60	55	42
- Severe symptoms **		1	2	0	0
· Mucositis					
- Any symptoms		17	35	21	25
- Severe Symptoms **		0	3	0	2

[a] Based on worst course analysis.

[b] TAXOL dose in mg/m ^{2} /infusion duration in hours.

[c] All patients received premedication.

** Severe events are defined as at least Grade III toxicity.

Myelosuppression was dose and schedule related, with the schedule effect being more prominent. The development of severe hypersensitivity reactions (HSRs) was rare; 1% of the patients and 0.2% of the courses overall. There was no apparent dose or schedule effect seen for the HSRs. Peripheral neuropathy was clearly dose-related, but schedule did not appear to affect the incidence.

Breast: For the 458 patients who received single-agent TAXOL in the Phase 3 breast carcinoma study, the following table shows the incidence of important adverse events by treatment arm (each arm was administered by a 3-hour infusion).

Table 11: Frequency [a] of Important Adverse Events in the Phase 3 Breast Carcinoma Study

		Percent of Patients	
		175/3 [b] (n=229)	135/3 [b] (n=229)
Bone Marrow			
- Neutropenia	< 2,000/mm ^{3} < 500/mm ^{3}	90 28	81 19
- Thrombocytopenia	< 100,000/mm ^{3} < 50,000/mm ^{3}	11 3	7 2
- Anemia	< 11 g/dL < 8 g/dL	55 4	47 2
- Infections		23	15
- Febrile Neutropenia		2	2
Hypersensitivity Reaction [c]			
- All		36	31
- Severe **		0	<1
Peripheral Neuropathy			
- Any symptoms		70	46
- Severe symptoms **		7	3
Mucositis			
- Any symptoms		23	17
- Severe symptoms **		3	<1

[a] Based on worst course analysis.

[b] TAXOL dose in mg/m ^{2} /infusion duration in hours.

[c] All patients received premedication.

** Severe events are defined as at least Grade III toxicity.

Myelosuppression and peripheral neuropathy were dose related. There was one severe hypersensitivity reaction (HSR) observed at the dose of 135 mg/m ^{2} .

First-Line NSCLC in Combination: In the study conducted by the Eastern Cooperative Oncology Group (ECOG), patients were randomized to either TAXOL (T) 135 mg/m ^{2} as a 24-hour infusion in combination with cisplatin (c) 75 mg/m ^{2} , TAXOL (T) 250 mg/m ^{2} as a 24-hour infusion in combination with cisplatin (c) 75mg/m ^{2} with G-CSF support, or cisplatin (c) 75 mg/m ^{2} on day 1, followed by etoposide (VP) 100 mg/m ^{2} on days 1, 2 and 3 (control).

The following table shows the incidence of important adverse events.

Table 12: Frequency [a] of Important Adverse Events in the Phase 3 Study for First-Line NSCLC

		Percent of Patients		
		T135/24 [b] c75 (n=195)	**T250/24 [c] c75 (n=197)**	**VP100 [d] c75 (n=196)**
· Bone Marrow				
- Neutropenia	< 2,000/mm ^{3} < 500/mm ^{3}	89 74 [e]	86 65	84 55
- Thrombocytopenia	< normal < 50,000/mm ^{3}	48 6	68 12	62 16
- Anemia	< normal < 8 g/dL	94 22	96 19	95 28
- Infections		38	31	
· Hypersensitivity Reaction [f]				
- All		**16**	**27**	**13**
- Severe **		**1**	**4**	**1**
· Arthralgia/Myalgia				
- Any symptoms		21 [e]	42 [e]	9
- Severe symptoms **		3	11	1
· Nausea/Vomiting				
- Any symptoms		85	87	81
- Severe symptoms **		27	29	22
· Mucositis				
- Any symptoms		**18**	**28**	**16**
- Severe symptoms **		**1**	**4**	**2**
· Neuromotor Toxicity				
- Any symptoms		**37**	**47**	**44**
- Severe symptoms **		6	12	7
· Neurosensory Toxicity				
- Any symptoms		**48**	61	25
- Severe symptoms **		**13**	28 [e]	8
· Cardiovascular Events				
- Any symptoms		**33**	**39**	**24**
- Severe symptoms **		13	12	8

[a] Based on worst course analysis.

[b] TAXOL dose in mg/m ^{2} /infusion duration in hours; cisplatin dose in mg/m ^{2}.

[c] TAXOL dose in mg/m ^{2} /infusion duration in hours with G-CSF support; cisplatin dose in mg/m ^{2}.

[d] Etoposide (VP) dose in mg/m ^{2} was administered I.V. on days 1, 2 and 3; cisplatin dose in mg/m ^{2}.

[e] $p<0.05$

[f] All patients received premedication.

** Severe events are defined as at least Grade III toxicity.

Toxicity was generally more severe in the high-dose TAXOL (paclitaxel) Injection treatment arm (T250/c75) than in the low-dose TAXOL arm (T135/c75). Compared to the cisplatin/etoposide arm,

patients in the low-dose TAXOL arm experienced more arthralgia/myalgia of any grade and more severe neutropenia. The incidence of febrile neutropenia was not reported in this study.

Kaposi's Sarcoma: The following table shows the frequency of important adverse events in the 85 patients with KS treated with two different single-agent TAXOL regimens.

Table 13: Frequency [a] of Important Adverse Events in the AIDS-Related Kaposi's Sarcoma Studies

		Percent of Patients	
		Study CA139-174 TAXOL 135/3 [b] q 3 wk (n=29)	Study CA139-281 TAXOL 100/3 [b] q 2 wk (n=56)
- Bone Marrow			
- Neutropenia	< 2,000/mm ^{3} < 500/mm ^{3}	100 76	95 35
- Thrombocytopenia	< 100,000/mm ^{3} < 50,000/mm ^{3}	52 17	27 5
- Anemia	< 11 g/dL < 8 g/dL	86 34	73 25
- Febrile Neutropenia		55	9
- Opportunistic Infection			
- Any		76	54
- Cytomegalovirus		45	27
- Herpes Simplex		38	11
- *Pneumocystis carinii*		14	21
- *M. avium-intracellulare*		24	4
- Candidiasis, esophageal		7	9
- Cryptosporidiosis		7	7
- Cryptococcal meningitis		3	2
- Leukoencephalopathy		---	2
- Hypersensitivity Reaction [c]			
- All		14	9
- Cardiovascular			
- Hypotension		17	9
- Bradycardia		3	---
- Peripheral Neuropathy			
- Any		79	46
- Severe **		10	2
- Myalgia/Arthralgia			
- Any		93	48
- Severe **		14	16
- Gastrointestinal			
- Nausea and Vomiting		69	70

- Diarrhea		90	73
- Mucositis		45	20
Renal (creatinine elevation)			
- Any		34	18
- Severe **		7	5
Discontinuation for drug toxicity		7	[illegible]
[a] Based on worst course analysis.			
[b] TAXOL dose in mg/m ^{2} /infusion duration in hours.			
[c] All patients received premedication.			
** Severe events are defined as at least Grade III toxicity.			

As demonstrated in this table, toxicity was more pronounced in the study utilizing TAXOL at a dose of 135 mg/m ^{2} every 3 weeks than in the study utilizing TAXOL at a dose of 100 mg/m ^{2} every 2 weeks. Notably, severe neutropenia (76% versus 35%), febrile neutropenia (55% versus 9%), and opportunistic infections (76% versus 54%) were more common with the former dose and schedule. The differences between the two studies with respect to dose escalation and use of hematopoietic growth factors, as described above, should be taken into account. (See **CLINICAL STUDIES: AIDS-Related Kaposi's Sarcoma** section.) Note also that only 26% of the 85 patients in these studies received concomitant treatment with protease inhibitors, whose effect on paclitaxel metabolism has not yet been studied.

Adverse Event Experiences by Body System: Unless otherwise noted, the following discussion refers to the overall safety database of 812 patients with solid tumors treated with single-agent TAXOL in clinical studies. Toxicities that occurred with greater severity or frequency in previously untreated patients with ovarian carcinoma or NSCLC who received TAXOL in combination with cisplatin and that occurred with a difference that was clinically significant in this population are also described. The frequency and severity of important adverse events for the Phase 3 first- and second-line ovarian, breast carcinoma, NSCLC, and Kaposi's sarcoma studies are presented above in tabular form by treatment arm. In addition, rare events have been reported from postmarketing experience or from other clinical studies. The frequency and severity of adverse events have been generally similar for patients receiving TAXOL for the treatment of ovarian, breast, or lung carcinoma or Kaposi's sarcoma, but patients with AIDS-related Kaposi's sarcoma may have more frequent and severe hematologic toxicity, infections, and febrile neutropenia. These patients require a lower dose intensity and supportive care. (See **CLINICAL STUDIES: Aids-Related Kaposi's Sarcoma** section.) Toxicities that were observed only in or were noted to have occurred with greater severity in the population with Kaposi's sarcoma and that occurred with a difference that was clinically significant in this population are described.

Hematologic: Bone marrow suppression was the major dose-limiting toxicity of TAXOL. Neutropenia, the most important hematologic toxicity, was dose and schedule dependent and was generally rapidly reversible. Among patients treated in the Phase 3 second-line ovarian study with a 3-hour infusion, neutrophil counts declined below 500 cells/mm ^{3} in 13% of the patients treated with a dose of 135 mg/m ^{2} compared to 27% at a dose of 175 mg/m ^{2} (p=0.05). In the same study, severe neutropenia (<500 cells/mm ^{3}) was more frequent with the 24-hour than with the 3-hour infusion; infusion duration had a greater impact on myelosuppression than dose. Neutropenia did not appear to increase with cumulative exposure and did not appear to be more frequent nor more severe for patients previously treated with radiation therapy.

In the study where TAXOL was administered to patients with ovarian carcinoma at a dose of 135 mg/m ^{2} /24 hours in combination with cisplatin versus the control arm of cyclophosphamide plus cisplatin, the incidences of grade IV neutropenia and of febrile neutropenia were significantly greater in the TAXOL plus cisplatin arm than in the control arm. Grade IV neutropenia occurred in 81% on the TAXOL plus cisplatin arm versus 58% on the cyclophosphamide plus cisplatin arm, and febrile neutropenia occurred in 15% and 4% respectively. On the TAXOL/cisplatin arm, there were 35/1074 (3%) courses with fever

in which Grade IV neutropenia was reported at some time during the course. When TAXOL followed by cisplatin was administered to patients with advanced NSCLC in the ECOG study, the incidences of Grade IV neutropenia were 74% (TAXOL 135 mg/m ^{2} /24 hours followed by cisplatin) and 65% (TAXOL 250 mg/m ^{2} /24 hours followed by cisplatin and G-CSF) compared with 55% in patients who received cisplatin/etoposide.

Fever was frequent (12% of all treatment courses). Infectious episodes occurred in 30% of all patients and 9% of all courses; these episodes were fatal in 1% of all patients, and included sepsis, pneumonia and peritonitis. In the Phase 3 second-line ovarian study, infectious episodes were reported in 19% of the patients given either 135 or 175 mg/m ^{2} dose by a 3-hour infusion. Urinary tract infections and upper respiratory tract infections were the most frequently reported infectious complications. In the immunosuppressed patient population with advanced HIV disease and poor-risk AIDS-related Kaposi's sarcoma, 61% of the patients reported at least one opportunistic infection. (See **CLINICAL STUDIES: AIDS-Related Kaposi's Sarcoma** section.) The use of supportive therapy, including G-CSF, is recommended for patients who have experienced severe neutropenia. (See **DOSAGE AND ADMINISTRATION** section.)

Thrombocytopenia was uncommon, and almost never severe (<50,000 cells/mm ^{3}). Twenty percent of the patients experienced a drop in their platelet count below 100,000 cells/mm ^{3} at least once while on treatment; 7% had a platelet count <50,000 cells/mm ^{3} at the time of their worst nadir. Bleeding episodes were reported in 4% of all courses and by 14% of all patients but most of the hemorrhagic episodes were localized and the frequency of these events was unrelated to the TAXOL (paclitaxel) Injection dose and schedule. In the Phase 3 second-line ovarian study, bleeding episodes were reported in 10% of the patients; no patients treated with the 3-hour infusion received platelet transfusions. In the Phase 3 NSCLC study, severe thrombocytopenia was experienced by 6% of the patients in the TAXOL 135 mg/m ^{2} /24 hours followed by cisplatin arm.

Anemia (Hb <11 g/dL) was observed in 78% of all patients and was severe (Hb <8 g/dL) in 16% of the cases. No consistent relationship between dose or schedule and the frequency of anemia was observed. Among all patients with normal baseline hemoglobin, 69% became anemic on study but only 7% had severe anemia. Red cell transfusions were required in 25% of all patients and in 12% of those with normal baseline hemoglobin levels.

Hypersensitivity Reactions (HSRs): All patients received premedication prior to TAXOL (see **WARNINGS** and **PRECAUTIONS: Hypersensitivity Reactions** sections). The frequency and severity of HSRs were not affected by the dose or schedule of TAXOL administration. In the Phase 3 second-line ovarian study, the 3-hour infusion was not associated with a greater increase in HSRs when compared to the 24-hour infusion. Hypersensitivity reactions were observed in 20% of all courses and in 41% of all patients. These reactions were severe in less than 2% of the patients and 1% of the courses. No severe reactions were observed after course 3 and severe symptoms occurred generally within the first hour of TAXOL infusion. The most frequent symptoms observed during these severe reactions were dyspnea, flushing, chest pain and tachycardia.

The minor hypersensitivity reactions consisted mostly of flushing (28%), rash (12%), hypotension (4%), dyspnea (2%), tachycardia (2%) and hypertension (1%). The frequency of hypersensitivity reactions remained relatively stable during the entire treatment period.

Rare reports of chills and reports of back pain in association with hypersensitivity reactions have been received as part of the continuing surveillance of TAXOL safety.

Cardiovascular: Hypotension, during the first 3 hours of infusion, occurred in 12% of all patients and 3% of all courses administered. Bradycardia, during the first 3 hours of infusion, occurred in 3% of all patients and 1% of all courses. In the Phase 3 second-line ovarian study, neither dose nor schedule had an effect on the frequency of hypotension and bradycardia. These vital sign changes most often caused no symptoms and required neither specific therapy nor treatment discontinuation. The frequency of hypotension and bradycardia were not influenced by prior anthracycline therapy.

Significant cardiovascular events possibly related to single-agent TAXOL occurred in approximately 1% of all patients. These events included syncope, rhythm abnormalities, hypertension and venous thrombosis. One of the patients with syncope treated with TAXOL at 175 mg/m ^{2} over 24 hours had progressive hypotension and died. The arrhythmias included asymptomatic ventricular tachycardia, bigeminy and complete AV block requiring pacemaker placement. Among patients with NSCLC treated with TAXOL in combination with cisplatin in the Phase 3 study, significant cardiovascular events occurred in 12%-13%. This apparent increase in cardiovascular events is possibly due to an increase in cardiovascular risk factors in patients with lung cancer.

Electrocardiogram (ECG) abnormalities were common among patients at baseline. ECG abnormalities on study did not usually result in symptoms, were not dose-limiting, and required no intervention. ECG abnormalities were noted in 23% of all patients. Among patients with a normal ECG prior to study entry 14% of all patients developed an abnormal tracing while on study. The most frequently reported ECG modifications were non-specific repolarization abnormalities, sinus bradycardia, sinus tachycardia and premature beats. Among patients with normal ECGs at baseline, prior therapy with anthracyclines did not influence the frequency of ECG abnormalities.

Cases of myocardial infarction have been reported rarely. Congestive heart failure has been reported typically in patients who have received other chemotherapy, notably anthracyclines. (See **PRECAUTIONS: Drug Interactions** section.)

Rare reports of atrial fibrillation and supraventricular tachycardia have been received as part of the continuing surveillance of TAXOL safety.

Respiratory: Rare reports of interstitial pneumonia, lung fibrosis and pulmonary embolism have been received as part of the continuing surveillance of TAXOL safety. Rare reports of radiation pneumonitis have been received in patients receiving concurrent radiotherapy.

Neurologic: The frequency and severity of neurologic manifestations were dose-dependent, but were not influenced by infusion duration. Peripheral neuropathy was observed in 60% of all patients (3% severe) and in 52% (2% severe) of the patients without pre-existing neuropathy.

The frequency of peripheral neuropathy increased with cumulative dose. Neurologic symptoms were observed in 27% of the patients after the first course of treatment and in 34%-51% from course 2 to 10.

Peripheral neuropathy was the cause of TAXOL discontinuation in 1% of all patients. Sensory symptoms have usually improved or resolved within several months of TAXOL discontinuation. The incidence of neurologic symptoms did not increase in the subset of patients previously treated with cisplatin. Pre-existing neuropathies resulting from prior therapies are not a contraindication for TAXOL therapy. In patients with NSCLC, administration of TAXOL followed by cisplatin resulted in greater incidence of severe neurotoxicity compared to the incidence in patients with ovarian or breast cancer treated with single-agent TAXOL. Severe neurosensory symptoms were noted in 13% of NSCLC patients receiving TAXOL 135 mg/m ^{2} by 24-infusion followed by cisplatin 75 mg/m ^{2} and 8% of NSCLC patients receiving cisplatin/etoposide (see Table 12).

Other than peripheral neuropathy, serious neurologic events following TAXOL administration have been rare (<1%) and have included grand mal seizures, syncope, ataxia and neuroencephalopathy.

Rare reports of autonomic neuropathy resulting in paralytic ileus have been received as part of the continuing surveillance of TAXOL safety. Optic nerve and/or visual disturbances (scintillating scotomata) have also been reported, particularly in patients who have received higher doses than those recommended. These effects generally have been reversible. However, rare reports in the literature of abnormal visual evoked potentials in patients have suggested persistent optic nerve damage.

Arthralgia/Myalgia: There was no consistent relationship between dose or schedule of TAXOL and

the frequency or severity of arthralgia/myalgia. Sixty percent of all patients treated experienced arthralgia/myalgia; 8% experienced severe symptoms. The symptoms were usually transient, occurred two or three days after TAXOL administration, and resolved within a few days. The frequency and severity of musculoskeletal symptoms remained unchanged throughout the treatment period.

Hepatic: No relationship was observed between liver function abnormalities and either dose or schedule of TAXOL (paclitaxel) Injection administration. Among patients with normal baseline liver function 7%, 22% and 19% had elevations in bilirubin, alkaline phosphatase and AST (SGOT), respectively. Prolonged exposure to TAXOL was not associated with cumulative hepatic toxicity.

Rare reports of hepatic necrosis and hepatic encephalopathy leading to death have been received as part of the continuing surveillance of TAXOL safety.

Renal: Among the patients treated for Kaposi's sarcoma with TAXOL, five patients had renal toxicity of grade III or IV severity. One patient with suspected HIV nephropathy of grade IV severity had to discontinue therapy. The other four patients had renal insufficiency with reversible elevations of serum creatinine.

Gastrointestinal (GI): Nausea/vomiting, diarrhea and mucositis were reported by 52%, 38% and 31% of all patients, respectively. These manifestations were usually mild to moderate. Mucositis was schedule dependent and occurred more frequently with the 24-hour than with the 3-hour infusion.

In patients with poor-risk AIDS-related Kaposi's sarcoma, nausea/vomiting, diarrhea, and mucositis were reported by 69%, 79%, and 28% of patients, respectively. One third of patients with Kaposi's sarcoma complained of diarrhea prior to study start. (See **CLINICAL STUDIES: AIDS-Related Kaposi's Sarcom** a section.)

In the first-line Phase 3 ovarian carcinoma study, the incidence of nausea and vomiting when TAXOL was administered in combination with cisplatin appeared to be greater compared with the database for single-agent TAXOL in ovarian and breast carcinoma. In the same study, diarrhea of any grade was reported more frequently (16%) compared to the control arm (8%) ($p=0.008$), but there was no difference for severe diarrhea.

Rare reports of intestinal obstruction, intestinal perforation, pancreatitis, ischemic colitis, and dehydration have been received as part of the continuing surveillance of TAXOL safety. Rare reports of neutropenic enterocolitis (typhlitis), despite the coadministration of G-CSF, were observed in patients treated with TAXOL alone and in combination with other chemotherapeutic agents.

Injection Site Reaction: Injection site reactions, including reactions secondary to extravasation, were usually mild and consisted of erythema, tenderness, skin discoloration, or swelling at the injection site. These reactions have been observed more frequently with the 24-hour infusion than with the 3-hour infusion. Recurrence of skin reactions at a site of previous extravasation following administration of TAXOL at a different site, i.e., "recall", has been reported rarely.

Rare reports of more severe events such as phlebitis, cellulitis, induration, skin exfoliation, necrosis and fibrosis have been received as part of the continuing surveillance of TAXOL safety. In some cases the onset of the injection site reaction either occurred during a prolonged infusion or was delayed by a week to ten days.

A specific treatment for extravasation reactions is unknown at this time. Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration.

Other Clinical Events: Alopecia was observed in almost all (87%) of the patients. Transient skin changes due to TAXOL-related hypersensitivity reactions have been observed, but no other skin toxicities were significantly associated with TAXOL administration. Nail changes (changes in pigmentation or discoloration of nail bed) were uncommon (2%). Edema was reported in 21% of all

patients (17% of those without baseline edema); only 1% had severe edema and none of these patients required treatment discontinuation. Edema was most commonly focal and disease-related. Edema was observed in 5% of all courses for patients with normal baseline and did not increase with time on study

Rare reports of skin abnormalities related to radiation recall as well as reports of maculopapular rash and pruritus have been received as part of the continuing surveillance of TAXOL safety.

Reports of asthenia and malaise have been received as part of the continuing surveillance of TAXOL safety. In the Phase 3 trial of TAXOL 135 mg/m ^{2} over 24 hours in combination with cisplatin as first-line therapy of ovarian cancer, asthenia was reported in 17% of the patients, significantly greater than the 10% incidence observed in the control arm of cyclophosphamide/cisplatin.

Accidental Exposure: Upon inhalation, dyspnea, chest pain, burning eyes, sore throat and nausea have been reported. Following topical exposure, events have included tingling, burning and redness.

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OVERDOSAGE

There is no known antidote for TAXOL overdosage. The primary anticipated complications of overdosage would consist of bone marrow suppression, peripheral neurotoxicity and mucositis. Overdoses in pediatric patients may be associated with acute ethanol toxicity (see **PRECAUTIONS Pediatric Use** section).

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DOSAGE AND ADMINISTRATION

Note: Contact of the undiluted concentrate with plasticized PVC equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP [di-(2-ethylhexyl)phthalate], which may be leached from PVC infusion bags or sets, diluted TAXOL solutions should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

All patients should be premedicated prior to TAXOL administration in order to prevent severe hypersensitivity reactions. Such premedication may consist of dexamethasone 20 mg PO administered approximately 12 and 6 hours before TAXOL, diphenhydramine (or its equivalent) 50 mg I.V. 30 to 60 minutes prior to TAXOL, and cimetidine (300 mg) or ranitidine (50 mg) I.V. 30 to 60 minutes before TAXOL.

For patients with **carcinoma of the ovary,** the following regimens are recommended

1. For previously untreated patients with carcinoma of the ovary, the recommended regimen, given every 3 weeks, is TAXOL administered intravenously over 24 hours at a dose of 135 mg/m ^{2} followed by cisplatin at a dose of 75 mg/m ^{2} .
2. In patients previously treated with chemotherapy for carcinoma of the ovary, TAXOL has been used at several doses and schedules; however, the optimal regimen is not yet clear (see **CLINICAL STUDIES: Ovarian Carcinoma** section). The recommended regimen is TAXOL 135 mg/m ^{2} or 175 mg/m ^{2} administered intravenously over 3 hours every 3 weeks.

For patients with **carcinoma of the breast,** TAXOL at a dose of 175 mg/m ^{2} administered intravenously over 3 hours every 3 weeks has been shown to be effective after failure of chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. (See **CLINICAL STUDIES: Breast Carcinoma** section.)

For patients with **non-small cell lung carcinoma** , the recommended regimen, given every 3 weeks, is TAXOL administered intravenously over 24 hours at a dose of 135 mg/m ^{2} followed by cisplatin, 75 mg/m ^{2} .

For patients with **AIDS-related Kaposi's sarcoma,** TAXOL administered at a dose of 135 mg/m ^{2} given intravenously over 3 hours every 3 weeks or at a dose of 100 mg/m ^{2} given intravenously over 3 hours every 2 weeks is recommended (dose intensity 45-50 mg/m ^{2} /week). In the two clinical trials evaluating these schedules (see **CLINICAL STUDIES: AIDS-Related Kaposi's Sarcoma** section), the former schedule (135 mg/m ^{2} every 3 weeks) was more toxic than the latter. In addition, all patients with low performance status were treated with the latter schedule (100 mg/m ^{2} every 2 weeks).

Based upon the immunosuppression in patients with advanced HIV disease, the following modifications are recommended in these patients:

1. Reduce the dose of dexamethasone as one of the three premedication drugs to 10 mg PO (instead of 20 mg PO);
2. Initiate or repeat treatment with TAXOL (paclitaxel) Injection only if the neutrophil count is at least 1000 cells/mm ^{3} ;
3. Reduce the dose of subsequent courses of TAXOL by 20% for patients who experience severe neutropenia (neutrophil <500 cells/mm ^{3} for a week or longer); and
4. Initiate concomitant hematopoietic growth factor (G-CSF) as clinically indicated.

For the therapy of patients with solid tumors (ovary, breast and NSCLC), courses of TAXOL should not be repeated until the neutrophil count is at least 1,500 cells/mm ^{3} and the platelet count is at least 100,000 cells/mm ^{3} . TAXOL should not be given to patients with AIDS-related Kaposi's sarcoma if the baseline or subsequent neutrophil count is less than 1000 cells/mm ^{3} . Patients who experience severe neutropenia (neutrophil <500 cells/mm ^{3} for a week or longer) or severe peripheral neuropathy during TAXOL therapy should have dosage reduced by 20% for subsequent courses of TAXOL. The incidence of neurotoxicity and the severity of neutropenia increase with dose.

Preparation and Administration Precautions: TAXOL is a cytotoxic anticancer drug and, as with other potentially toxic compounds, caution should be exercised in handling TAXOL. The use of gloves is recommended. If TAXOL solution contacts the skin, wash the skin immediately and thoroughly with soap and water. Following topical exposure, events have included tingling, burning and redness. If TAXOL contacts mucous membranes, the membranes should be flushed thoroughly with water. Upon inhalation, dyspnea, chest pain, burning eyes, sore throat, and nausea have been reported.

Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration. (See **PRECAUTIONS: Injection Site Reaction** section.)

Preparation for Intravenous Administration: TAXOL must be diluted prior to infusion. TAXOL should be diluted in 0.9% Sodium Chloride Injection, USP; 5% Dextrose Injection, USP; 5% Dextrose and 0.9% Sodium Chloride Injection, USP or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2 mg/mL. The solutions are physically and chemically stable for up to 27 hours at ambient temperature (approximately 25°C) and room lighting conditions. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

Upon preparation, solutions may show haziness, which is attributed to the formulation vehicle. No significant losses in potency have been noted following simulated delivery of the solution through i.v. tubing containing an in-line (0.22 micron) filter.

Data collected for the presence of the extractable plasticizer DEHP [di-(2-ethylhexyl)phthalate] show that levels increase with time and concentration when dilutions are prepared in PVC containers. Consequently, the use of plasticized PVC containers and administration sets is not recommended. TAXOL solutions should be prepared and stored in glass, polypropylene, or polyolefin containers. Non-PVC containing administration sets, such as those which are polyethylene-lined, should be used.

TAXOL should be administered through an in-line filter with a microporous membrane not greater than 0.22 microns. Use of filter devices such as IVEX-2® filters which incorporate short inlet and outlet PVC-coated tubing has not resulted in significant leaching of DEHP.

The Chemo Dispensing Pin™ device or similar devices with spikes should not be used with vials of TAXOL since they can cause the stopper to collapse resulting in loss of sterile integrity of the TAXOL solution.

Stability: Unopened vials of TAXOL Injection are stable until the date indicated on the package when stored between 20°-25°C (68°-77°F), in the original package. Neither freezing nor refrigeration adversely affects the stability of the product. Upon refrigeration components in the TAXOL vial may precipitate, but will redissolve upon reaching room temperature with little or no agitation. There is no impact on product quality under these circumstances. If the solution remains cloudy or if an insoluble precipitate is noted, the vial should be discarded. Solutions for infusion prepared as recommended are stable at ambient temperature (approximately 25°C) and lighting conditions for up to 27 hours.

IVEX-2® is the registered trademark of the Millipore Corporation.

Chemo Dispensing Pin™ is a trademark of B. Braun Medical Incorporated.

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HOW SUPPLIED

NDC 0015-3475-30	30 mg/5 mL multidose vial individually packaged in a carton.
NDC 0015-3476-30	100 mg/16.7 mL multidose vial individually packaged in a carton.
NDC 0015-3479-11	300 mg/50 mL multidose vial individually packaged in a carton.

Storage: Store the vials in original cartons between 20°-25°C (68°-77°F). Retain in the original package to protect from light.

Handling and Disposal: Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published. [1-7] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

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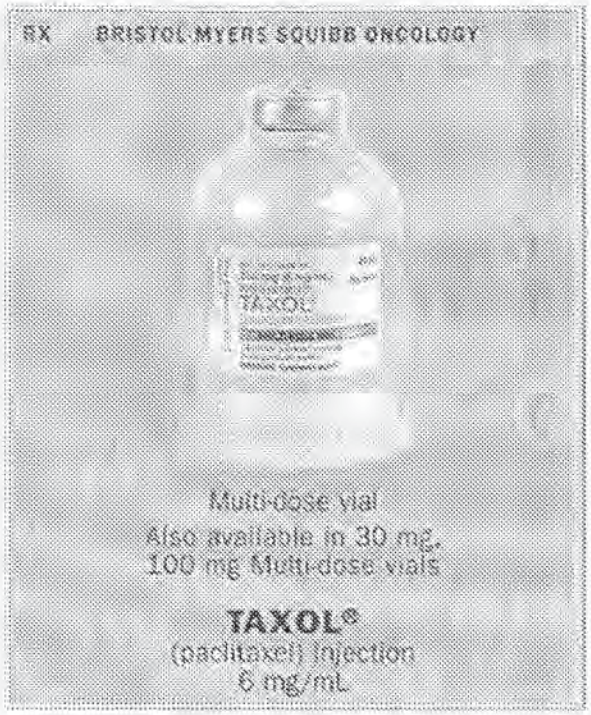
PRODUCT PHOTO(S):

NOTE: These photos can be used only for identification by shape, color, and imprint. They do not depict actual *or relative* size.

The product samples shown here have been supplied by the manufacturer and reproduced in full color by PDR as a quick-reference identification aid. While every effort has been made to assure accurate reproduction, please remember that any visual identification should be considered preliminary. In cases of poisoning or suspected overdosage, the drug's identity should be verified by chemical analysis.



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51-006186-04 Revised December 1998

PDR® entry for
Taxotere for Injection Concentrate (Aventis)

Description

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WARNING

TAXOTERE® (docetaxel) for Injection Concentrate should be administered under the supervision of a qualified physician experienced in the use of antineoplastic agents. Appropriate management of complications is possible only when adequate diagnostic and treatment facilities are readily available.

The incidence of treatment-related mortality associated with TAXOTERE therapy is increased in patients with abnormal liver function, in patients receiving higher doses, and in patients with non-small cell lung carcinoma and a history of prior treatment with platinum-based chemotherapy who receive TAXOTERE at a dose of 100 mg/m ^{2} (see **WARNINGS**).

TAXOTERE should generally not be given to patients with bilirubin > upper limit of normal (ULN), or to patients with SGOT and/or SGPT >1.5 × ULN concomitant with alkaline phosphatase > 2.5 × ULN. Patients with elevations of bilirubin or abnormalities of transaminase concurrent with alkaline phosphatase are at increased risk for the development of grade 4 neutropenia, febrile neutropenia, infections, severe thrombocytopenia, severe stomatitis, severe skin toxicity, and toxic death. Patients with isolated elevations of transaminase > 1.5 × ULN also had a higher rate of febrile neutropenia grade 4 but did not have an increased incidence of toxic death. Bilirubin, SGOT or SGPT, and alkaline phosphatase values should be obtained prior to each cycle of TAXOTERE therapy and reviewed by the treating physician.

TAXOTERE therapy should not be given to patients with neutrophil counts of < 1500 cells/mm ^{3}. In order to monitor the occurrence of neutropenia, which may be severe and result in infection, frequent blood cell counts should be performed on all patients receiving TAXOTERE.

Severe hypersensitivity reactions characterized by hypotension and/or bronchospasm, or generalized rash/erythema occurred in 2.2% (2/92) of patients who received the recommended 3-day dexamethasone premedication. Hypersensitivity reactions requiring discontinuation of the TAXOTERE infusion were reported in five patients who did not receive premedication. These reactions resolved after discontinuation of the infusion and the administration of appropriate therapy. TAXOTERE must not be given to patients who have a history of severe hypersensitivity reactions to TAXOTERE or to other drugs formulated with polysorbate 80 (see **WARNINGS**).

Severe fluid retention occurred in 6.5% (6/92) of patients despite use of a 3-day

Severe fluid retention occurred in 6.5% (6/92) of patients despite use of a 3-day dexamethasone premedication regimen. It was characterized by one or more of the following events: poorly tolerated peripheral edema, generalized edema, pleural effusion requiring urgent drainage, dyspnea at rest, cardiac tamponade, or pronounced abdominal distention (due to ascites) (see **PRECAUTIONS**).

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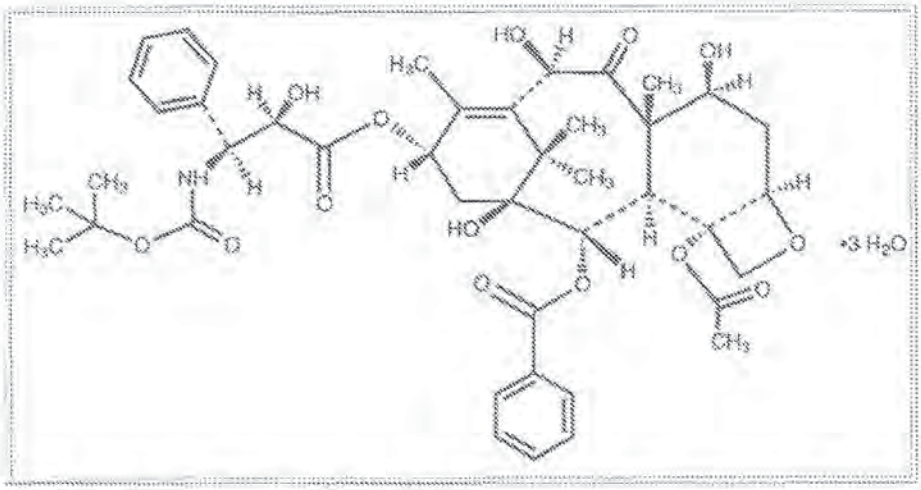
DESCRIPTION

Docetaxel is an antineoplastic agent belonging to the taxoid family. It is prepared by semisynthesis beginning with a precursor extracted from the renewable needle biomass of yew plants. The chemical name for docetaxel is (2R,3S)-N-carboxy-3-phenylisoserine,N- *tert* -butyl ester, 13-ester with 5(beta)-20-epoxy-1,2(alpha),4,7(beta),10(beta),13(alpha)-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate. Docetaxel has the following structural formula:



Docetaxel is a white to almost-white powder with an empirical formula of $C_{43}H_{53}NO_{14} \cdot 3H_2O$, and a molecular weight of 861.9. It is highly lipophilic and practically insoluble in water. TAXOTERE (docetaxel) for Injection Concentrate is a clear yellow to brownish-yellow viscous solution. TAXOTERE is sterile, non-pyrogenic, and is available in single-dose vials containing 20 mg (0.5 mL) or 80 mg (2.0 mL) docetaxel (anhydrous). Each mL contains 40 mg docetaxel (anhydrous) and 1040 mg polysorbate 80.

TAXOTERE for Injection Concentrate requires dilution prior to use. A sterile, non-pyrogenic, single-dose diluent is supplied for that purpose. The diluent for TAXOTERE contains 13% ethanol in Water for Injection, and is supplied in 1.5 mL (to be used with 20 mg TAXOTERE for Injection Concentrate) and 6.0 mL (to be used with 80 mg TAXOTERE for Injection Concentrate) vials.

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CLINICAL PHARMACOLOGY

Docetaxel is an antineoplastic agent that acts by disrupting the microtubular network in cells that is essential for mitotic and interphase cellular functions. Docetaxel binds to free tubulin and promotes the assembly of tubulin into stable microtubules while simultaneously inhibiting their disassembly. This leads to the production of microtubule bundles without normal function and to the stabilization of microtubules, which results in the inhibition of mitosis in cells. Docetaxel's binding to microtubules does not alter the number of protofilaments in the bound microtubules, a feature which differs from most spindle poisons currently in clinical use.

HUMAN PHARMACOKINETICS

The pharmacokinetics of docetaxel have been evaluated in cancer patients after administration of 20-115 mg/m ^{2} in phase I studies. The area under the curve (AUC) was dose proportional following doses of 70-115 mg/m ^{2} with infusion times of 1 to 2 hours. Docetaxel's pharmacokinetic profile is consistent with a three-compartment pharmacokinetic model, with half-lives for the (alpha), (beta), and (gamma) phases of 4 min, 36 min, and 11.1 hr, respectively. The initial rapid decline represents distribution to the peripheral compartments and the late (terminal) phase is due, in part, to a relatively slow efflux of docetaxel from the peripheral compartment. Mean values for total body clearance and steady state volume of distribution were 21 L/h/m ^{2} and 113 L, respectively. Mean total body clearance for Japanese patients dosed at the range of 10-90 mg/m ^{2} was similar to that of European/American populations dosed at 100 mg/m ^{2}, suggesting no significant difference in the elimination of docetaxel in the two populations.

A study of ^{14}C-docetaxel was conducted in three cancer patients. Docetaxel was eliminated in both the urine and feces following oxidative metabolism of the *tert* -butyl ester group, but fecal excretion was the main elimination route. Within 7 days, urinary and fecal excretion accounted for approximately 6% and 75% of the administered radioactivity, respectively. About 80% of the radioactivity recovered in feces is excreted during the first 48 hours as 1 major and 3 minor metabolites with very small amounts (less than 8%) of unchanged drug.

A population pharmacokinetic analysis was carried out after TAXOTERE treatment of 535 patients dosed at 100 mg/m ^{2}. Pharmacokinetic parameters estimated by this analysis were very close to those estimated from phase I studies. The pharmacokinetics of docetaxel were not influenced by age or gender and docetaxel total body clearance was not modified by pretreatment with dexamethasone. In patients with clinical chemistry data suggestive of mild to moderate liver function impairment (SGOT and/or SGPT >1.5 times the upper limit of normal [ULN] concomitant with alkaline phosphatase >2.5 times ULN), total body clearance was lowered by an average of 27%, resulting in a 38% increase in systemic exposure (AUC). This average, however, includes a substantial range and there is, at present, no measurement that would allow recommendation for dose adjustment in such patients. Patients with combined abnormalities of transaminase and alkaline phosphatase should, in general, not be treated with TAXOTERE.

In vitro studies showed that docetaxel is about 94% protein bound, mainly to (alpha) $_1$ -acid glycoprotein, albumin, and lipoproteins. In three cancer patients, the *in vitro* binding to plasma proteins was found to be approximately 97%. Dexamethasone does not affect the protein binding of docetaxel.

In vitro drug interaction studies revealed that docetaxel is metabolized by the CYP3A4 isoenzyme, and its metabolism can be inhibited by CYP3A4 inhibitors, such as ketoconazole, erythromycin, troleandomycin, and nifedipine. Based on *in vitro* findings, it is likely that CYP3A4 inhibitors and/or substrates may lead to substantial increases in docetaxel blood concentrations. No clinical studies have been performed to evaluate this finding (see **PRECAUTIONS**).

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CLINICAL STUDIES

Breast Cancer: The efficacy and safety of TAXOTERE have been evaluated in locally advanced or metastatic breast cancer after failure of previous chemotherapy (alkylating agent-containing regimens or anthracycline-containing regimens), primarily at a dose of 100 mg/m ^{2} given as a 1-hour infusion every 3 weeks, but with some experience at 60 mg/m ^{2}, in two large randomized trials and a number of smaller single arm studies.

Randomized Trials: In one randomized trial, patients with a history of prior treatment with an anthracycline-containing regimen were assigned to treatment with TAXOTERE or the combination of

mitomycin (12 mg/m ^{2} every 6 weeks) and vinblastine (6 mg/m ^{2} every 3 weeks). 203 patients were randomized to TAXOTERE and 189 to the comparator arm. Most patients had received prior chemotherapy for metastatic disease; only 27 patients on the TAXOTERE arm and 33 patients on the comparator arm entered the study following relapse after adjuvant therapy. Three-quarters of patients had measurable, visceral metastases. The primary endpoint was time to progression. The following table summarizes the study results:

Efficacy of TAXOTERE in the Treatment of Breast Cancer Patients Previously Treated with an Anthracycline-Containing Regimen (Intent-to-Treat Analysis)

Efficacy Parameter	Docetaxel (n=203)	Mitomycin/ Vinblastine (n=189)	p-value
Median Survival		8.7 months	
Risk Ratio *, Mortality (Docetaxel: Control)	0.73		p=0.01 Log Rank
95% CI (Risk Ratio)	0.58-0.93		
Median Time to Progression	4.3 months	2.5 months	
Risk Ratio *, Progression (Docetaxel: Control)	0.75		p=0.01 Log Rank
95% CI (Risk Ratio)	0.61-0.94		
Overall Response Rate	28.1%	9.5%	p<0.0001 Chi Square
Complete Response Rate	3.4%	1.6%	

* For the risk ratio, a value less than 1.00 favors docetaxel.

In a second randomized trial, patients previously treated with an alkylating-containing regimen were assigned to treatment with TAXOTERE or doxorubicin (75 mg/m ^{2} every 3 weeks). 161 patients were randomized to TAXOTERE and 165 patients to doxorubicin. Approximately one-half of patients had received prior chemotherapy for metastatic disease, and one-half entered the study following relapse after adjuvant therapy. Three-quarters of patients had measurable, visceral metastases. The primary endpoint was time to progression. The study results are summarized below:

Efficacy of TAXOTERE in the Treatment of Breast Cancer Patients Previously Treated with an Alkylating-Containing Regimen (Intent-to-Treat Analysis)

Efficacy Parameter	Docetaxel (n=161)	Doxorubicin (n=165)	p-value
Median Survival	14.7 months	14.3 months	
Risk Ratio *, Mortality (Docetaxel: Control)	0.89		p=0.39 Log Rank
95% CI (Risk Ratio)	0.68-1.16		
Median Time to Progression	6.5 months	5.3 months	
Risk Ratio *, Progression (Docetaxel: Control)	0.93		p=0.45 Log Rank
95% CI (Risk Ratio)	0.71-1.16		
Overall Response Rate	45.3%	29.7%	p=0.004
Complete Response Rate	6.8%	4.2%	Chi Square

* For the risk ratio, a value less than 1.00 favors docetaxel.

Single Arm Studies: TAXOTERE at a dose of 100 mg/m ^{2} was studied in six single arm studies involving a total of 309 patients with metastatic breast cancer in whom previous chemotherapy had failed. Among these, 190 patients had anthracycline-resistant breast cancer, defined as progression during an anthracycline-containing chemotherapy regimen for metastatic disease, or relapse during an anthracycline-containing adjuvant regimen. In anthracycline-resistant patients, the overall response rate was 37.9% (72/190; 95% C.I.: 31.0-44.8) and the complete response rate was 2.1%.

TAXOTERE was also studied in three single arm Japanese studies at a dose of 60 mg/m ^{2} , in 174 patients who had received prior chemotherapy for locally advanced or metastatic breast cancer. Among 26 patients whose best response to an anthracycline had been progression, the response rate was 34.6% (95% C.I.: 17.2-55.7), similar to the response rate in single arm studies of 100 mg/m ^{2} .

Hematologic and Other Toxicity: Relation to dose and baseline liver chemistry abnormalities. Hematologic and other toxicity is increased at higher doses and in patients with elevated baseline liver function tests (LFTs). In the following tables, adverse drug reactions are compared for three populations: 730 patients with normal LFTs given TAXOTERE at 100 mg/m ^{2} in the randomized and single arm studies of metastatic breast cancer after failure of previous chemotherapy; 18 patients in these studies who had abnormal baseline LFTs (defined as SGOT and/or SGPT > 1.5 times ULN concurrent with alkaline phosphatase > 2.5 times ULN); and 174 patients in Japanese studies given TAXOTERE at 60 mg/m ^{2} who had normal LFTs.

Hematologic Adverse Events in Breast Cancer Patients Previously Treated with Chemotherapy Treated at TAXOTERE 100 mg/m ^{2} with Normal or Elevated Liver Function Tests or 60 mg/m ^{2} with Normal Liver Function Tests

	TAXOTERE 100 mg/m ^{2}		TAXOTERE 60 mg/m ^{2}
Adverse Event	Normal LFTs * n=730 %	Elevated LFTs ** n=18 %	Normal LFTs * n=174 %
Neutropenia			
Any <2000 cells/mm ^{3}	98.4	100	95.4
Grade 4 <500 cells/mm ^{3}	84.4	93.8	74.9
Thrombocytopenia			
Any <100,000 cells/mm ^{3}	10.8	44.4	14.4
Grade 4 <20,000 cells/mm ^{3}	0.6	16.7	1.1
Anemia <11 g/dL	94.6	94.4	64.9
Infection ***			
Any	22.5	38.9	1.1
Grade 3 and 4	7.1	33.3	0
Febrile Neutropenia ****			
By Patient	11.8	33.3	0
By Course	2.4	8.6	0
Septic Death	1.5	5.6	1.1
Non-Septic Death	1.1	11.1	0

* Normal Baseline LFTs: Transaminases </= 1.5 times ULN or alkaline phosphatase </= 2.5 times ULN or isolated elevations of transaminases or alkaline phosphatase up to 5 times ULN

** Elevated Baseline LFTs: SGOT and/or SGPT >1.5 times ULN concurrent with alkaline phosphatase >2.5 times ULN

*** Incidence of infection requiring hospitalization and/or intravenous antibiotics was 8.5% (n=62) among the 730 patients with normal LFTs at baseline; 7 patients had concurrent grade 3 neutropenia, and 46 patients had grade 4 neutropenia.

**** Febrile Neutropenia: For 100 mg/m ^{2}, ANC grade 4 and fever > 38°C with IV antibiotics and/or hospitalization; for 60 mg/m ^{2}, ANC grade 3/4 and fever > 38.1°C

Non-Hematologic Adverse Events in Breast Cancer Patients Previously Treated with Chemotherapy Treated at TAXOTERE 100 mg/m ^{2} with Normal or Elevated Liver Function Tests or 60 mg/m ^{2} with Normal Liver Function Tests

	TAXOTERE 100 mg/m ^{2}		TAXOTERE 60 mg/m ^{2}
Adverse Event	Normal LFTs * n=730 %	Elevated LFTs ** n=18 %	Normal LFTs * n=174 %
Acute Hypersensitivity			
Reaction Regardless of Premedication			
Any	13.0	5.6	0.6
Severe	1.2	0	0
Fluid Retention ***			
Regardless of Premedication			
Any	56.2	61.1	12.6
Severe	7.9	16.7	0
Neurosensory			
Any	56.8	50	19.5
Severe	5.8	0	0
Myalgia	22.7	33.3	3.4
Cutaneous			
Any	44.8	61.1	30.5
Severe	4.8	16.7	0
Asthenia			
Any	65.2	44.4	65.5
Severe	16.6	22.2	0
Diarrhea			
Any	42.2	27.8	NA
Severe	6.3	11.1	
Stomatitis			
Any	53.3	66.7	19.0
Severe	7.8	38.9	0.6

*** Normal Baseline LFTs: Transaminases </= 1.5 times ULN or alkaline phosphatase </= 2.5 times ULN or isolated elevations of transaminases or alkaline phosphatase up to 5 times ULN**

**** Elevated Baseline Liver Function: SGOT and/or SGPT >1.5 times ULN concurrent with alkaline phosphatase >2.5 times ULN**

***** Fluid Retention includes (by COSTART): edema (peripheral, localized, generalized, lymphedema, pulmonary edema, and edema otherwise not specified) and effusion (pleural, pericardial, and ascites); no premedication given with the 60 mg/m ^{2} dose**
NA = not available

Non-Small Cell Lung Cancer (NSCLC): The efficacy and safety of TAXOTERE in non-small cell lung cancer have been evaluated in patients with locally advanced or metastatic disease and a history of prior treatment with a platinum-based chemotherapy regimen. Two randomized, controlled trials

established that a TAXOTERE dose of 75 mg/m ^{2} was tolerable and yielded a favorable outcome (see below). TAXOTERE at a dose of 100 mg/m ^{2}, however, was associated with unacceptable hematologic toxicity, infections, and treatment-related mortality and this dose should not be used (see **BOXED WARNING**, **WARNINGS**, and **DOSAGE AND ADMINISTRATION** sections).

One trial (TAX317), randomized patients with locally advanced or metastatic non-small cell lung cancer, a history of prior platinum-based chemotherapy, no history of taxane exposure, and an ECOG performance status </=2 to TAXOTERE or best supportive care. The primary endpoint of the study was survival. Patients were initially randomized to TAXOTERE 100 mg/m ^{2} or best supportive care, but early toxic deaths at this dose led to a dose reduction to TAXOTERE 75 mg/m ^{2}. A total of 104 patients were randomized in this amended study to either TAXOTERE 75 mg/m ^{2} or best supportive care.

In a second randomized trial (TAX320), 373 patients with locally advanced or metastatic non-small cell lung cancer, a history of prior platinum-based chemotherapy, and an ECOG performance status </=2 were randomized to TAXOTERE 75 mg/m ^{2}, TAXOTERE 100 mg/m ^{2} and a treatment in which the investigator chose either vinorelbine 30 mg/m ^{2} days 1, 8, and 15 repeated every 3 weeks or ifosfamide 2 g/m ^{2} days 1-3 repeated every 3 weeks. Forty percent of the patients in this study had a history of prior paclitaxel exposure. The primary endpoint was survival in both trials. The efficacy data for the TAXOTERE 75 mg/m ^{2} arm and the comparator arms are summarized in the table below and in figures 1 and 2 showing the survival curves for the two studies.

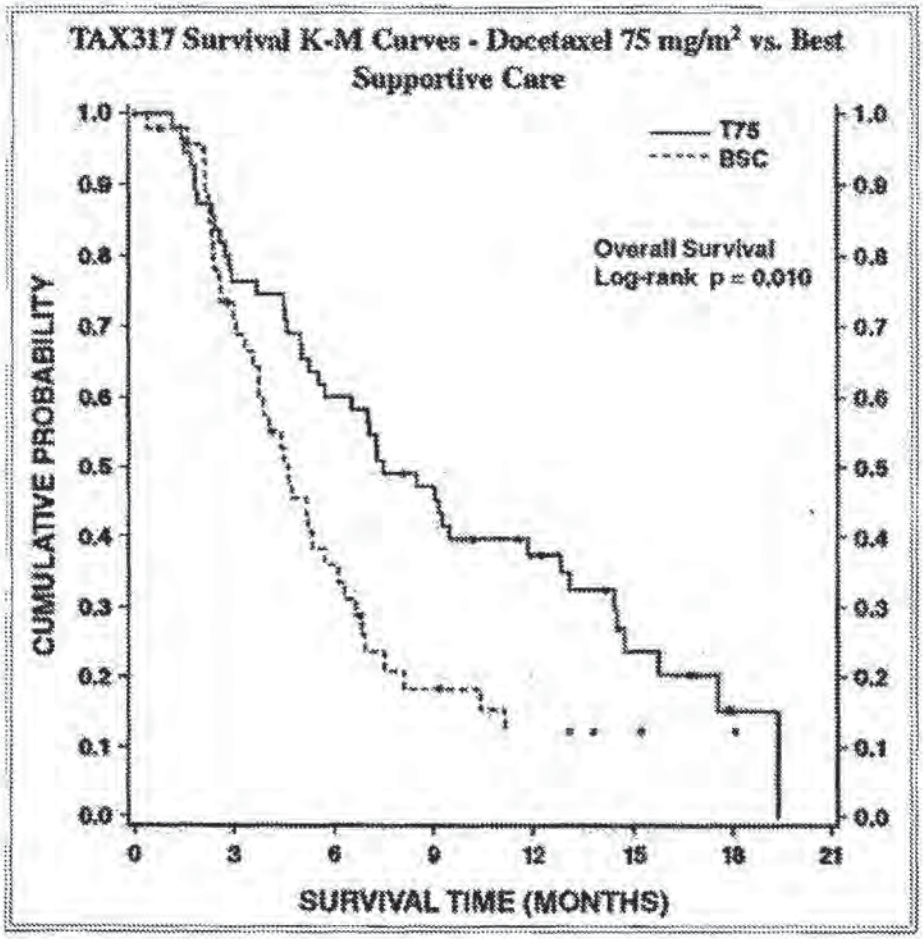
Efficacy of TAXOTERE in the Treatment of Non-Small Cell Lung Cancer Patients Previously Treated with a Platinum-Based Chemotherapy Regimen (Intent-to-Treat Analysis)

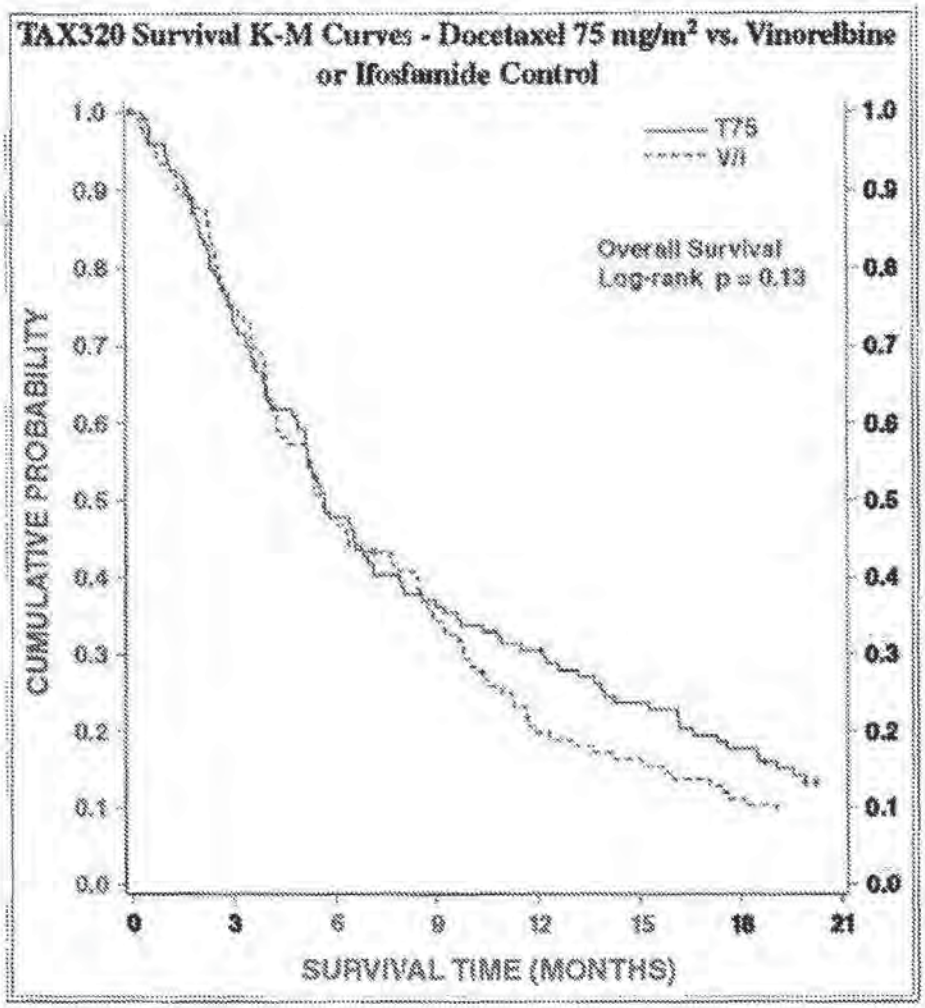
	TAX317		TAX320	
	Docetaxel 75 mg/m ^{2} n=55	Best Supportive Care/75 n=49	Docetaxel 75 mg/m ^{2} n=125	Control (V/I) n=123
Overall Survival Log-rank Test	p=0.01		p=0.13	
Risk Ratio ##, Mortality (Docetaxel: Control)	0.56		0.82	
95% CI (Risk Ratio)	(0.35, 0.88)		(0.63, 1.06)	
Median Survival	7.5 months *	4.6 months	5.7 months	5.6 months
95% CI	(5.5, 12.8)	(3.7, 6.1)	(5.1, 7.1)	(4.4, 7.9)
% 1-year Survival	37% * #	12%	30% * #	20%
95% CI	(24, 50)	(2, 23)	(22, 39)	(13, 27)
Time to Progression	12.3 weeks *	7.0 weeks	8.3 weeks	7.6 weeks
95% CI	(9.0, 18.3)	(6.0, 9.3)	(7.0, 11.7)	(6.7, 10.1)
Response Rate	5.5%	Not Applicable	5.7%	0.8%
95% CI	(1.1, 15.1)		(2.3, 11.3)	(0.0, 4.5)

* p</=0.05; # uncorrected for multiple comparisons; ## a value less than 1.00 favors docetaxel.

Only one of the two trials (TAX317) showed a clear effect on survival, the primary endpoint; that trial

also showed an increased rate of survival to one year. In the second study (TAX320) the rate of survival at one year favored TAXOTERE 75 mg/m ^{2} .





Patients treated with TAXOTERE at a dose of 75 mg/m ^{2} experienced no deterioration in performance status and body weight relative to the comparator arms used in these trials.

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INDICATIONS AND USAGE

Breast Cancer: TAXOTERE is indicated for the treatment of patients with locally advanced or metastatic breast cancer after failure of prior chemotherapy.

Non-Small Cell Lung Cancer: TAXOTERE is indicated for the treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of prior platinum-based chemotherapy.

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CONTRAINDICATIONS

TAXOTERE is contraindicated in patients who have a history of severe hypersensitivity reactions to docetaxel or to other drugs formulated with polysorbate 80.

TAXOTERE should not be used in patients with neutrophil counts of <1500 cells/mm ^{3}

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WARNINGS

TAXOTERE should be administered under the supervision of a qualified physician experienced in the use of antineoplastic agents. Appropriate management of complications is possible only when adequate diagnostic and treatment facilities are readily available.

Toxic Deaths

Breast Cancer: TAXOTERE administered at 100 mg/m ^{2} was associated with deaths considered possibly or probably related to treatment in 2.0% (19/965) of metastatic breast cancer patients, both previously treated and untreated, with normal baseline liver function and in 11.5% (7/61) of patients with various tumor types who had abnormal baseline liver function (SGOT and/or SGPT > 1.5 times ULN together with AP > 2.5 times ULN). Among patients dosed at 60 mg/m ^{2} , mortality related to treatment occurred in 0.6% (3/481) of patients with normal liver function, and in 3 of 7 patients with abnormal liver function. Approximately half of these deaths occurred during the first cycle. Sepsis accounted for the majority of the deaths.

Non-Small Cell Lung Cancer: TAXOTERE administered at a dose of 100 mg/m ^{2} in patients with locally advanced or metastatic non-small cell lung cancer who had a history of prior platinum-based chemotherapy was associated with increased treatment-related mortality (14% and 5% in two randomized, controlled studies). There were 2.8% treatment-related deaths among the 176 patients treated at the 75 mg/m ^{2} dose in the randomized trials. Among patients who experienced treatment-related mortality at the 75 mg/m ^{2} dose level, 3 of 5 patients had a PS of 2 at study entry (see **BOXED WARNING** , **CLINICAL STUDIES** , and **DOSAGE AND ADMINISTRATION** sections).

Premedication Regimen: All patients should be premedicated with oral corticosteroids such as dexamethasone 16 mg per day (*e.g.,* 8 mg BID) for 3 days starting 1 day prior to TAXOTERE to reduce the severity of fluid retention and hypersensitivity reactions (see **DOSAGE AND ADMINISTRATION** section). This regimen was evaluated in 92 patients with metastatic breast cancer previously treated with chemotherapy given TAXOTERE at a dose of 100 mg/m ^{2} every 3 weeks.

Hypersensitivity Reactions: Patients should be observed closely for hypersensitivity reactions, especially during the first and second infusions. Severe hypersensitivity reactions characterized by hypotension and/or bronchospasm, or generalized rash/erythema occurred in 2.2% of the 92 patients premedicated with 3-day corticosteroids. Hypersensitivity reactions requiring discontinuation of the TAXOTERE infusion were reported in 5 out of 1260 patients with various tumor types who did not receive premedication, but in 0/92 patients premedicated with 3-day corticosteroids. Patients with a history of severe hypersensitivity reactions should not be rechallenged with TAXOTERE.

Hematologic Effects: Neutropenia (< 2000 neutrophils/mm ^{3}) occurs in virtually all patients given 60-100 mg/m ^{2} of TAXOTERE and grade 4 neutropenia (< 500 cells/mm ^{3}) occurs in 85% of patients given 100 mg/m ^{2} and 75% of patients given 60 mg/m ^{2} . Frequent monitoring of blood counts is, therefore, essential so that dose can be adjusted. TAXOTERE should not be administered to patients with neutrophils < 1500 cells/mm ^{3} .

Febrile neutropenia occurred in about 12% of patients given 100 mg/m ^{2} but was very uncommon in patients given 60 mg/m ^{2} . Hematologic responses, febrile reactions and infections, and rates of septic death for different regimens are dose related and are described in **CLINICAL STUDIES** .

Three breast cancer patients with severe liver impairment (bilirubin > 1.7 times ULN) developed fatal gastrointestinal bleeding associated with severe drug-induced thrombocytopenia.

Hepatic Impairment: (see **BOXED WARNING**)

Fluid Retention: (see **BOXED WARNING**

Pregnancy: TAXOTERE can cause fetal harm when administered to pregnant women. Studies in both rats and rabbits at doses >/= 0.3 and 0.03 mg/kg/day, respectively (about 1/50 and 1/300 the daily maximum recommended human dose on a mg/m ^{2} basis), administered during the period of organogenesis, have shown that TAXOTERE is embryotoxic and fetotoxic (characterized by intrauterine mortality, increased resorption, reduced fetal weight, and fetal ossification delay). The doses indicated above also caused maternal toxicity.

There are no adequate and well-controlled studies in pregnant women using TAXOTERE. If TAXOTERE is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus or potential risk for loss of the pregnancy. Women of childbearing potential should be advised to avoid becoming pregnant during therapy with TAXOTERE.

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PRECAUTIONS

General: Responding patients may not experience an improvement in performance status on therapy and may experience worsening. The relationship between changes in performance status, response to therapy, and treatment-related side effects has not been established.

Hematologic Effects: In order to monitor the occurrence of myelotoxicity, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving TAXOTERE. Patients should not be retreated with subsequent cycles of TAXOTERE until neutrophils recover to a level > 1500 cells/mm ^{3} and platelets recover to a level > 100,000 cells/mm ^{3} .

A 25% reduction in the dose of TAXOTERE® (docetaxel) for Injection Concentrate is recommended during subsequent cycles following severe neutropenia (< 500 cells/mm ^{3}) lasting 7 days or more, febrile neutropenia, or a grade 4 infection in a TAXOTERE cycle (see **DOSAGE AND ADMINISTRATION** section).

Hypersensitivity Reactions: Hypersensitivity reactions may occur within a few minutes following initiation of a TAXOTERE infusion. If minor reactions such as flushing or localized skin reactions occur, interruption of therapy is not required. More severe reactions, however, require the immediate discontinuation of TAXOTERE and aggressive therapy. All patients should be premedicated with an oral corticosteroid prior to the initiation of the infusion of TAXOTERE (see **BOXED WARNING** and **WARNINGS : Premedication Regimen**).

Cutaneous: Localized erythema of the extremities with edema followed by desquamation has been observed. In case of severe skin toxicity, an adjustment in dosage is recommended (see **DOSAGE AND ADMINISTRATION** section). The discontinuation rate due to skin toxicity was 1.6% (15/965) for metastatic breast cancer patients. Among 92 breast cancer patients premedicated with 3-day corticosteroids, there were no cases of severe skin toxicity reported and no patient discontinued TAXOTERE due to skin toxicity.

Fluid Retention: Severe fluid retention has been reported following TAXOTERE therapy (see **BOXED WARNING** and **WARNINGS : Premedication Regimen**). Patients should be premedicated with oral corticosteroids prior to each TAXOTERE administration to reduce the incidence and severity of fluid retention (see **DOSAGE AND ADMINISTRATION** section). Patients with pre-existing effusions should be closely monitored from the first dose for the possible exacerbation of the effusions.

When fluid retention occurs, peripheral edema usually starts in the lower extremities and may become

generalized with a median weight gain of 2 kg.

Among 92 breast cancer patients premedicated with 3-day corticosteroids, moderate fluid retention occurred in 27.2% and severe fluid retention in 6.5%. The median cumulative dose to onset of moderate or severe fluid retention was 819 mg/m ^{2} . 9.8% (9/92) of patients discontinued treatment due to fluid retention: 4 patients discontinued with severe fluid retention; the remaining 5 had mild or moderate fluid retention. The median cumulative dose to treatment discontinuation due to fluid retention was 1021 mg/m ^{2} . Fluid retention was completely, but sometimes slowly, reversible with a median of 16 weeks from the last infusion of TAXOTERE to resolution (range: 0 to 42 + weeks). Patients developing peripheral edema may be treated with standard measures, *e.g.*, salt restriction, oral diuretic(s).

Neurologic: Severe neurosensory symptoms (paresthesia, dysesthesia, pain) were observed in 5.5% (53/965) of metastatic breast cancer patients, and resulted in treatment discontinuation in 6.1%. When these symptoms occur, dosage must be adjusted. If symptoms persist, treatment should be discontinued (see **DOSAGE AND ADMINISTRATION** section). Patients who experienced neurotoxicity in clinical trials and for whom follow-up information on the complete resolution of the event was available had spontaneous reversal of symptoms with a median of 9 weeks from onset (range: 0 to 106 weeks). Severe peripheral motor neuropathy mainly manifested as distal extremity weakness occurred in 4.4% (42/965).

Asthenia: Severe asthenia has been reported in 14.9% (144/965) of metastatic breast cancer patients but has led to treatment discontinuation in only 1.8%. Symptoms of fatigue and weakness may last a few days up to several weeks and may be associated with deterioration of performance status in patients with progressive disease.

Information for Patients: For additional information, see the accompanying Patient Information Leaflet.

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Drug Interactions: There have been no formal clinical studies to evaluate the drug interactions of TAXOTERE with other medications. *In vitro* studies have shown that the metabolism of docetaxel may be modified by the concomitant administration of compounds that induce, inhibit, or are metabolized by cytochrome P450 3A4, such as cyclosporine, terfenadine, ketoconazole, erythromycin, and troleandomycin. Caution should be exercised with these drugs when treating patients receiving TAXOTERE as there is a potential for a significant interaction.

Carcinogenicity, Mutagenicity, Impairment of Fertility: No studies have been conducted to assess the carcinogenic potential of TAXOTERE. TAXOTERE has been shown to be clastogenic in the *in vitro* chromosome aberration test in CHO-K $_1$ cells and in the *in vivo* micronucleus test in the mouse, but it did not induce mutagenicity in the Ames test or the CHO/HGPRT gene mutation assays. TAXOTERE produced no impairment of fertility in rats when administered in multiple IV doses of up to 0.3 mg/kg (about 1/50 the recommended human dose on a mg/m ^{2} basis), but decreased testicular weights were reported. This correlates with findings of a 10-cycle toxicity study (dosing once every 21 days for 6 months) in rats and dogs in which testicular atrophy or degeneration was observed at IV doses of 5 mg/kg in rats and 0.375 mg/kg in dogs (about 1/3 and 1/15 the recommended human dose on a mg/m ^{2} basis, respectively). An increased frequency of dosing in rats produced similar effects at lower dose levels.

Pregnancy: Pregnancy Category D (see **WARNINGS** section).

Nursing Mothers: It is not known whether TAXOTERE is excreted in human milk. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants from TAXOTERE, mothers should discontinue nursing prior to taking the drug.

Pediatric Use: The safety and effectiveness of TAXOTERE in pediatric patients have not been established.

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ADVERSE REACTIONS

The adverse reactions are described separately for TAXOTERE 100 mg/m ^{2}, the maximum dose approved for breast cancer, and 75 mg/m ^{2}, the dose approved for advanced non-small cell lung carcinoma after prior platinum-based chemotherapy.

TAXOTERE 100 mg/m ^{2}: Adverse drug reactions occurring in at least 5% of patients are compared for three populations who received TAXOTERE administered at 100 mg/m ^{2} as a 1-hour infusion every 3 weeks: 2045 patients with various tumor types and normal baseline liver function tests; the subset of 965 patients with locally advanced or metastatic breast cancer, both previously treated and untreated with chemotherapy, who had normal baseline liver function tests; and an additional 61 patients with various tumor types who had abnormal liver function tests at baseline. These reactions were described using COSTART terms and were considered possibly or probably related to TAXOTERE. At least 95% of these patients did not receive hematopoietic support. The safety profile is generally similar in patients receiving TAXOTERE for the treatment of breast cancer and in patients with other tumor types.

Summary of Adverse Events in Patients Receiving TAXOTERE at 100 mg/m ^{2}

Adverse Event	All Tumor Types Normal LFTs * n=2045 %	All Tumor Types Elevated LFTs ** n=61 %	Breast Cancer Normal LFTs * n=965 %
Hematologic			
Neutropenia			
<2000 cells/mm ^{3}	95.5	96.4	98.5
<500 cells/mm ^{3}	75.4	87.5	85.9
Leukopenia			
<4000 cells/mm ^{3}	95.6	98.3	98.6
<1000 cells/mm ^{3}	31.6	46.6	43.7
Thrombocytopenia			
<100,000 cells/mm ^{3}	8.0	24.6	9.2
Anemia			
<11 g/dL	90.4	91.8	93.6
<8 g/dL	8.8	31.1	7.7
Febrile Neutropenia ***	11.0	26.2	12.3
Septic Death	1.6	4.9	
Non-Septic Death	0.6	6.6	
Infections			
Any	21.6	32.8	22.2
Severe	6.1	16.4	6.4
Fever in Absence of Infection			
Any	31.2	41.0	35.1

Severe	2.1	8.2	2.2
Hypersensitivity Reactions			
Regardless of Premedication			
Any	21.0	19.7	17.6
Severe	4.2	9.8	2.6
With 3-day Premedication	n=92	n=3	n=92
Any	15.2	33.3	15.2
Severe	2.2	0	2.2
Fluid Retention			
Regardless of Premedication			
Any	47.0	39.3	59.7
Severe	6.9	8.2	8.9
With 3-day Premedication	n=92	n=3	n=92
Any	64.1	66.7	64.1
Severe	6.5	33.3	6.5
Neurosensory			
Any	49.3	34.4	58.3
Severe	4.3	0	5.5
Cutaneous			
Any	47.6	54.1	47.0
Severe	4.8	9.8	5.2
Nail Changes			
Any	30.6	23.0	40.5
Severe	2.5	4.9	3.7
Gastrointestinal			
Nausea	38.8	37.7	42.1
Vomiting	22.3	23.0	23.4
Diarrhea	38.7	32.8	42.6
Severe	4.7	4.9	5.5
Stomatitis			
Any	41.7	49.2	51.7
Severe	5.5	13.0	7.4
Alopecia	75.8	62.3	74.2
Asthenia			
Any	61.8	52.5	66.3
Severe	12.8	24.6	14.9
Myalgia			
Any	18.9	16.4	21.1
Severe	1.5	1.6	1.8
Arthralgia	9.2	6.6	8.2
Infusion Site Reactions	4.4	3.3	4.0

*** Normal Baseline LFTs: Transaminases </= 1.5 times ULN or alkaline phosphatase </= 2.5 times ULN or isolated elevations of transaminases or alkaline phosphatase up to 5 times ULN**

**** Elevated Baseline LFTs: SGOT and/or SGPT >1.5 times ULN concurrent with alkaline phosphatase >2.5 times ULN**

***** Febrile Neutropenia: ANC grade 4 with fever > 38°C with IV antibiotics and/or hospitalization**

Hematologic: (see **WARNINGS**). Reversible marrow suppression was the major dose-limiting toxicity of TAXOTERE. The median time to nadir was 7 days, while the median duration of severe neutropenia (<500 cells/mm ^{3}) was 7 days. Among 2045 patients with solid tumors and normal baseline LFTs, severe neutropenia occurred in 75.4% and lasted for more than 7 days in 2.9% of cycles.

Febrile neutropenia (<500 cells/mm ^{3} with fever > 38°C with IV antibiotics and/or hospitalization) occurred in 11% of patients with solid tumors, in 12.3% of patients with metastatic breast cancer, and in 9.8% of 92 breast cancer patients premedicated with 3-day corticosteroids.

Severe infectious episodes occurred in 6.1% of patients with solid tumors, in 6.4% of patients with metastatic breast cancer, and in 5.4% of 92 breast cancer patients premedicated with 3-day corticosteroids.

Thrombocytopenia (<100,000 cells/mm ^{3} associated with fatal gastrointestinal hemorrhage has been reported.

Hypersensitivity Reactions: Severe hypersensitivity reactions are discussed in the **BOXED WARNING** , **WARNINGS** , and **PRECAUTIONS** sections. Minor events, including flushing, rash with or without pruritus, chest tightness, back pain, dyspnea, drug fever, or chills, have been reported and resolved after discontinuing the infusion and appropriate therapy.

Fluid Retention: (see **BOXED WARNING** , **WARNINGS** : **Premedication Regimen** , and **PRECAUTIONS** sections).

Cutaneous: Severe skin toxicity is discussed in **PRECAUTIONS** . Reversible cutaneous reactions characterized by a rash including localized eruptions, mainly on the feet and/or hands, but also on the arms, face, or thorax, usually associated with pruritus, have been observed. Eruptions generally occurred within 1 week after TAXOTERE infusion, recovered before the next infusion, and were not disabling.

Severe nail disorders were characterized by hypo- or hyperpigmentation, and occasionally by onycholysis (in 0.8% of patients with solid tumors) and pain.

Neurologic: (see **PRECAUTIONS**

Gastrointestinal: Gastrointestinal reactions (nausea and/or vomiting and/or diarrhea) were generally mild to moderate. Severe reactions occurred in 3-5% of patients with solid tumors and to a similar extent among metastatic breast cancer patients. The incidence of severe reactions was 1% or less for the 92 breast cancer patients premedicated with 3-day corticosteroids.

Severe stomatitis occurred in 5.5% of patients with solid tumors, in 7.4% of patients with metastatic breast cancer, and in 1.1% of the 92 breast cancer patients premedicated with 3-day corticosteroids.

Cardiovascular: Hypotension occurred in 2.8% of patients with solid tumors; 1.2% required treatment. Clinically meaningful events such as heart failure, sinus tachycardia, atrial flutter, dysrhythmia, unstable angina, pulmonary edema, and hypertension occurred rarely. 8.1% (7/86) of metastatic breast cancer patients receiving TAXOTERE 100 mg/m ^{2} in a randomized trial and who had serial left ventricular ejection fractions assessed developed deterioration of LVEF by >/= 10% associated

with a drop below the institutional lower limit of normal

Infusion Site Reactions: Infusion site reactions were generally mild and consisted of hyperpigmentation, inflammation, redness or dryness of the skin, phlebitis, extravasation, or swelling of the vein.

Hepatic: In patients with normal LFTs at baseline, bilirubin values greater than the ULN occurred in 8.9% of patients. Increases in SGOT or SGPT > 1.5 times the ULN, or alkaline phosphatase > 2.5 times ULN, were observed in 18.9% and 7.3% of patients, respectively. While on TAXOTERE, increases in SGOT and/or SGPT > 1.5 times ULN concomitant with alkaline phosphatase > 2.5 times ULN occurred in 4.3% of patients with normal LFTs at baseline. (Whether these changes were related to the drug or underlying disease has not been established.)

TAXOTERE 75 mg/m ^{2} : Treatment emergent adverse drug reactions are shown below. Included in this table are safety data for a total of 176 patients with non-small cell lung carcinoma and a history of prior treatment with platinum-based chemotherapy who were treated in two randomized, controlled trials. These reactions were described using NCI Common Toxicity Criteria regardless of relationship to study treatment, except for the hematologic toxicities or otherwise noted.

Treatment Emergent Adverse Events in Non-Small Cell Lung Cancer Patients Receiving TAXOTERE Regardless of Relationship to Treatment *

Adverse Event	TAXOTERE 75 mg/m ^{2} n=176 %	Best Supportive Care n=49 %	Vinorelbine/ Ifosfamide n=119 %
Neutropenia			
Any	84.1	14.3	83.2
Grade 3/4	65.3	12.2	57.1
Leukopenia			
Any	83.5	6.1	89.1
Grade 3/4	49.4	0	42.9
Thrombocytopenia			
Any	8.0	0	7.6
Grade 3/4	2.8	0	1.7
Anemia			
Any	91.0	55.1	90.8
Grade 3/4	9.1	12.2	14.3
Febrile Neutropenia **	6.3	NA #	0.8
Infection			
Any	33.5	28.6	30.3
Grade 3/4	10.2	6.1	9.2
Treatment Related Mortality	2.8	NA #	3.4
Hypersensitivity Reactions			
Any	5.7	0	0.8
Grade 3/4	2.8	0	0

Fluid Retention			
Any	33.5	ND [#8]	22.7
Severe	2.8		3.4
Neurosensory			
Any	23.3	14.3	28.6
Grade 3/4	1.7	6.1	5.0
Neuromotor			
Any	15.9	8.2	10.1
Grade 3/4	4.5	6.1	3.4
Skin			
Any	19.9	6.1	16.8
Grade 3/4	0.6	2.0	0.8
Gastrointestinal			
Nausea			
Any	33.5	30.6	31.1
Grade 3/4	5.1	4.1	7.6
Vomiting			
Any	21.6	26.5	21.8
Grade 3/4	2.8	2.0	5.9
Diarrhea			
Any	22.7	6.1	11.8
Grade 3/4	2.8	0	4.2
Alopecia	56.3	34.7	49.6
Asthenia			
Any	52.8	57.1	53.8
Severe ***	18.2	38.8	22.7
Stomatitis			
Any	26.1	6.1	7.6
Grade 3/4	1.7	0	0.8
Pulmonary			
Any	40.9	49.0	45.4
Grade 3/4	21.0	28.6	18.5
Nail Disorder			
Any	11.4	0	1.7
Severe ***	1.1	0	0
Myalgia			
Any	6.3	0	2.5
Severe ***	0	0	0
Arthralgia			
Any	3.4	2.0	1.7
Severe ***	0	0	0.8

Taste Perversion			
Any	5.7	0	0
Severe ***	0.6	0	0
* Normal Baseline LFTs: Transaminases </= 1.5 times ULN or alkaline phosphatase </= 2.5 times ULN or isolated elevations of transaminases or alkaline phosphatase up to 5 times ULN			
** Febrile Neutropenia: ANC grade 4 with fever > 38°C with IV antibiotics and/or hospitalization			
*** COSTART term and grading system			
# Not Applicable; ## Not Done			

Ongoing Evaluation: The following serious adverse events of uncertain relationship to TAXOTERE have been reported:

Body as a whole: abdominal pain, diffuse pain, chest pain, radiation recall phenomenon

Cardiovascular: atrial fibrillation, deep vein thrombosis, ECG abnormalities, thrombophlebitis, pulmonary embolism, syncope, tachycardia, myocardial infarction

Digestive: constipation, duodenal ulcer, esophagitis, gastrointestinal hemorrhage, intestinal obstruction, ileus, gastrointestinal perforation, neutropenic enterocolitis, dehydration in relation to digestive disorders

Nervous: confusion, seizures

Respiratory: dyspnea, acute pulmonary edema, acute respiratory distress syndrome, interstitial pneumonia

Urogenital: renal insufficiency

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OVERDOSAGE

There is no known antidote for TAXOTERE overdosage. In case of overdosage, the patient should be kept in a specialized unit where vital functions can be closely monitored. Anticipated complications of overdosage include: bone marrow suppression, peripheral neurotoxicity, and mucositis. Patients should receive therapeutic G-CSF as soon as possible after discovery of overdose. Other appropriate symptomatic measures should be taken, as needed.

In two reports of overdose, one patient received 150 mg/m ^{2} and the other received 200 mg/m ^{2} as 1-hour infusions. Both patients experienced severe neutropenia, mild asthenia, cutaneous reactions, and mild paresthesia, and recovered without incident.

In mice, lethality was observed following single IV doses that were >/=154 mg/kg (about 4.5 times the recommended human dose on a mg/m ^{2} basis); neurotoxicity associated with paralysis, non-extension of hind limbs, and myelin degeneration was observed in mice at 48 mg/kg (about 1.5 times the recommended human dose on a mg/m ^{2} basis). In male and female rats, lethality was observed at a dose of 20 mg/kg (comparable to the recommended human dose on a mg/m ^{2} basis) and was associated with abnormal mitosis and necrosis of multiple organs.

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DOSAGE AND ADMINISTRATION

Breast Cancer: The recommended dose of TAXOTERE is 60-100 mg/m ^{2} administered intravenously over 1 hour every 3 weeks.

Non-Small Cell Lung Cancer: The recommended dose of TAXOTERE is 75 mg/m ^{2} administered intravenously over 1 hour every 3 weeks. A dose of 100 mg/m ^{2} in patients previously treated with chemotherapy was associated with increased hematologic toxicity, infection, and treatment-related mortality in randomized, controlled trials (see **BOXED WARNING**, **WARNINGS** and **CLINICAL STUDIES** sections).

Premedication Regimen: All patients should be premedicated with oral corticosteroids such as dexamethasone 16 mg per day (*e.g.*, 8 mg BID) for 3 days starting 1 day prior to TAXOTERE administration in order to reduce the incidence and severity of fluid retention as well as the severity of hypersensitivity reactions (see **BOXED WARNING**, **WARNINGS**, and **PRECAUTIONS** sections).

Dosage Adjustments During Treatment

Breast Cancer: Patients who are dosed initially at 100 mg/m ^{2} and who experience either febrile neutropenia, neutrophils < 500 cells/mm ^{3} for more than 1 week, or severe or cumulative cutaneous reactions during TAXOTERE therapy should have the dosage adjusted from 100 mg/m ^{2} to 75 mg/m ^{2}. If the patient continues to experience these reactions, the dosage should either be decreased from 75 mg/m ^{2} to 55 mg/m ^{2} or the treatment should be discontinued. Conversely, patients who are dosed initially at 60 mg/m ^{2} and who do not experience febrile neutropenia, neutrophils <500 cells/mm ^{3} for more than 1 week, severe or cumulative cutaneous reactions, or severe peripheral neuropathy during TAXOTERE therapy may tolerate higher doses. Patients who develop >/= grade 3 peripheral neuropathy should have TAXOTERE treatment discontinued entirely.

Non-Small Cell Lung Cancer: Patients who are dosed initially at 75 mg/m ^{2} and who experience either febrile neutropenia, neutrophils <500 cells/mm ^{3} for more than one week, severe or cumulative cutaneous reactions, or other grade 3/4 non-hematological toxicities during TAXOTERE treatment should have treatment withheld until resolution of the toxicity and then resumed at 55 mg/m ^{2}. Patients who develop >/= grade 3 peripheral neuropathy should have TAXOTERE treatment discontinued entirely.

Special Populations:

Hepatic Impairment: Patients with bilirubin > ULN should generally not receive TAXOTERE. Also, patients with SGOT and/or SGPT > 1.5 × ULN concomitant with alkaline phosphatase > 2.5 × ULN should generally not receive TAXOTERE.

Children: The safety and effectiveness of docetaxel in pediatric patients below the age of 16 years have not been established.

Elderly: No dosage adjustments are required for use in elderly.

PREPARATION AND ADMINISTRATION PRECAUTIONS

TAXOTERE is a cytotoxic anticancer drug and, as with other potentially toxic compounds, caution should be exercised when handling and preparing TAXOTERE solutions. The use of gloves is recommended. Please refer to **Handling and Disposal** section.

If TAXOTERE concentrate, initial diluted solution, or final dilution for infusion should come into contact with the skin, immediately and thoroughly wash with soap and water. If TAXOTERE concentrate, initial diluted solution, or final dilution for infusion should come into contact with mucosa, immediately and thoroughly wash with water.

TAXOTERE for Injection Concentrate requires two dilutions prior to administration. Please follow the preparation instructions provided below. **Note:** Both the TAXOTERE for Injection Concentrate and the diluent vials contain an overfill.

A. Preparation of the Initial Diluted Solution
   1. Remove the appropriate number of vials of TAXOTERE for Injection Concentrate and diluent (13% Ethanol in Water for Injection) from the refrigerator. Allow the vials to stand at room temperature for approximately 5 minutes.
   2. Aseptically withdraw the contents of the appropriate diluent vial into a syringe and transfer it to the appropriate vial of TAXOTERE for Injection Concentrate. **If the procedure is followed as described, an initial diluted solution of 10 mg docetaxel/mL will result.**
   3. Gently rotate the initial diluted solution for approximately 15 seconds to assure full mixture of the concentrate and diluent.
   4. The initial diluted TAXOTERE solution (10 mg docetaxel/mL) should be clear; however, there may be some foam on top of the solution due to the polysorbate 80. Allow the solution to stand for a few minutes to allow any foam to dissipate. It is not required that all foam dissipate prior to continuing the preparation process.
      The initial diluted solution may be used immediately or stored either in the refrigerator or at room temperature for a maximum of 8 hours.

B. Preparation of the Final Dilution for Infusion
   1. Aseptically withdraw the required amount of initial diluted TAXOTERE solution (10 mg docetaxel/mL) with a calibrated syringe and inject into a 250 mL infusion bag or bottle of either 0.9% Sodium Chloride solution or 5% Dextrose solution to produce a final concentration of 0.3 to 0.74 mg/mL.
      If a dose greater than 200 mg of TAXOTERE is required, use a larger volume of the infusion vehicle so that a concentration of 0.74 mg/mL TAXOTERE is not exceeded.
   2. Thoroughly mix the infusion by manual rotation.
   3. As with all parenteral products, TAXOTERE should be inspected visually for particulate matter or discoloration prior to administration whenever the solution and container permit. If the TAXOTERE for Injection initial diluted solution or final dilution for infusion is not clear or appears to have precipitation, these should be discarded.

The final TAXOTERE dilution for infusion should be administered intravenously as a 1-hour infusion under ambient room temperature and lighting conditions.
Contact of the TAXOTERE concentrate with plasticized PVC equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP (di-2-ethylhexyl phthalate), which may be leached from PVC infusion bags or sets, the final TAXOTERE dilution for infusion should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

Stability: TAXOTERE infusion solution, if stored between 2 and 25°C (36 and 77°F) is stable for 4 hours. Fully prepared TAXOTERE infusion solution (in either 0.9% Sodium Chloride solution or 5% Dextrose solution) should be used within 4 hours (including the 1 hour i.v. administration).

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HOW SUPPLIED

TAXOTERE for Injection Concentrate is supplied in a single-dose vial as a sterile, pyrogen-free, non-aqueous, viscous solution with an accompanying sterile, non-pyrogenic, diluent (13% ethanol in Water for Injection) vial. The following strengths are available:

TAXOTERE 80 MG (NDC 0075-8001-80)

TAXOTERE (docetaxel) 80 mg Concentrate for Infusion: 80 mg docetaxel in 2 mL polysorbate 80 and

diluent for TAXOTERE 80 mg. 13% (w/w) ethanol in Water for Injection. Both items are in a blister pack in one carton.

TAXOTERE 20 MG (NDC 0075-8001-20)

TAXOTERE (docetaxel) 20 mg Concentrate for Infusion: 20 mg docetaxel in 0.5 mL polysorbate 80 and diluent for TAXOTERE 20 mg. 13% (w/w) ethanol in Water for Injection. Both items are in a blister pack in one carton.

Storage: Store between 2 and 25°C (36 and 77°F). Retain in the original package to protect from bright light. Freezing does not adversely affect the product.

Handling and Disposal: Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published [1-7]. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

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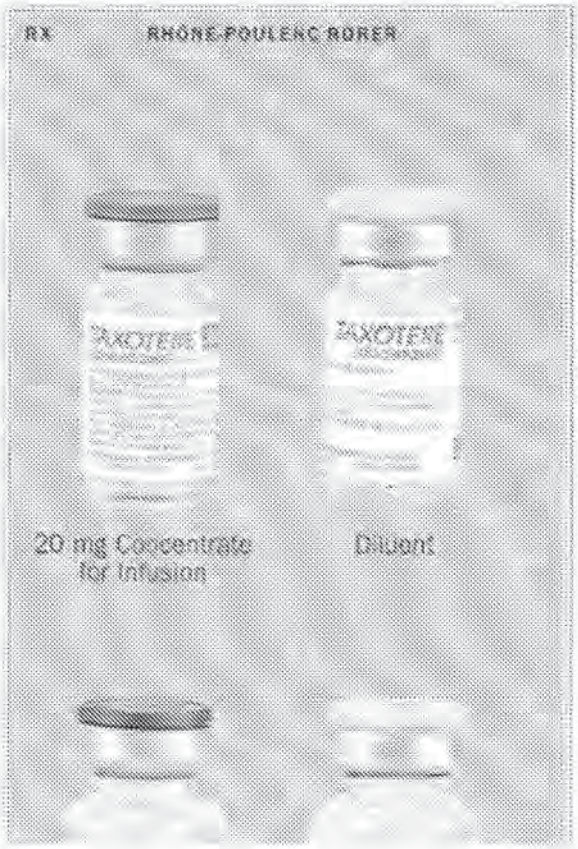
PRODUCT PHOTO(S):

NOTE: These photos can be used only for identification by shape, color, and imprint. They do not depict actual *or relative* size.

The product samples shown here have been supplied by the manufacturer and reproduced in full color by PDR as a quick-reference identification aid. While every effort has been made to assure accurate reproduction, please remember that any visual identification should be considered preliminary. In cases of poisoning or suspected overdosage, the drug's identity should be verified by chemical analysis.



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ORIGINAL REPORT

Randomized Phase II Trial of the Efficacy and Safety of Trastuzumab Combined With Docetaxel in Patients With Human Epidermal Growth Factor Receptor 2–Positive Metastatic Breast Cancer Administered As First-Line Treatment: The M77001 Study Group

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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ABSTRACT

Purpose
This randomized, multicenter trial compared first-line trastuzumab plus docetaxel versus docetaxel alone in patients with human epidermal growth factor receptor 2 (HER2) –positive metastatic breast cancer (MBC).

Patients and Methods
Patients were randomly assigned to six cycles of docetaxel 100 mg/m^2 every 3 weeks, with or without trastuzumab 4 mg/kg loading dose followed by 2 mg/kg weekly until disease progression.

Results
A total of 186 patients received at least one dose of the study drug. Trastuzumab plus docetaxel was significantly superior to docetaxel alone in terms of overall response rate (61% *v* 34%; $P = .0002$), overall survival (median, 31.2 *v* 22.7 months; $P = .0325$), time to disease progression (median, 11.7 *v* 6.1 months; $P = .0001$), time to treatment failure (median, 9.8 *v* 5.3 months; $P = .0001$), and duration of response (median, 11.7 *v* 5.7 months; $P = .009$). There was little difference in the number and severity of adverse events between the arms. Grade 3 to 4 neutropenia was seen more commonly with the combination (32%) than with docetaxel alone (22%), and there was a slightly higher incidence of febrile neutropenia in the combination arm (23% *v* 17%). One patient in the combination arm experienced symptomatic heart failure (1%). Another patient experienced symptomatic heart failure 5 months after discontinuation of trastuzumab because of disease progression, while being treated with an investigational anthracycline for 4 months.

Conclusion
Trastuzumab combined with docetaxel is superior to docetaxel alone as first-line treatment of patients with HER2-positive MBC in terms of overall survival, response rate, response duration, time to progression, and time to treatment failure, with little additional toxicity.

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INTRODUCTION

Human epidermal growth factor receptor 2 (HER2) is a key contributor to normal cell growth and differentiation.[1] However, when overexpressed, it is associated with neoplastic transformation of cells. Approximately 15% to 20% of breast cancers show HER2 overexpression (3+ by immunohistochemistry [IHC]) and/or amplification of the *HER2* gene. HER2-positive malignancies have a significantly more aggressive disease

course and lead to a worse clinical outcome, including shortened overall survival (OS), compared with those that do not overexpress HER2.[2,3] Trastuzumab is a humanized murine monoclonal antibody that binds specifically to the extracellular domain of the HER2 protein. The clinical efficacy and favorable safety profile of trastuzumab in metastatic breast cancer (MBC) have been demonstrated when administered as monotherapy[4,5] and in combination with the taxane paclitaxel.[6] Clinical benefits are greatest in patients with tumors strongly overexpressing HER2, graded 3+ by IHC, and/or with *HER2* gene amplification, as determined by fluorescence in situ hybridization (FISH). In a randomized phase III trial (study H0648g), which compared trastuzumab plus paclitaxel with paclitaxel alone, the median OS in patients with IHC 3+ disease was 40% longer in the combination arm than in the paclitaxel-alone arm (24.8 *v* 17.9 months),[6,7] highlighting the benefit of combining trastuzumab with paclitaxel.

Docetaxel is another widely used taxane and has a similar mechanism of action to that of paclitaxel. It is one of the most active chemotherapeutic agents used in the treatment of MBC.[8,9] Preclinical data indicate synergy between docetaxel and trastuzumab,[10] and clinical activity has been confirmed in a number of phase II studies, with response rates of 44% to 83% and toxicity comparable with that of single-agent docetaxel.[11-22]

The preclinical data, the known clinical activity of trastuzumab plus paclitaxel, and the favorable results of phase II trials of trastuzumab plus docetaxel provided a strong rationale for investigating trastuzumab and docetaxel in a randomized trial. Study M77001 was designed to compare the overall response rate (ORR) of trastuzumab plus docetaxel versus docetaxel alone. The trial also aimed to characterize the safety profile of trastuzumab in combination with docetaxel versus docetaxel alone, and to compare duration of response (DR), time to disease progression (TTP), time to treatment failure (TTF), and OS in the two treatment arms.

Patients and Methods

Study Design

M77001 is an open-label, comparative, randomized, multicenter, multinational trial comparing the efficacy and safety of first-line trastuzumab (Herceptin, F. Hoffmann-La Roche, Basel, Switzerland) plus docetaxel (Taxotere, Aventis Pharma, Strasbourg, France) with docetaxel alone in patients with HER2-positive MBC. Patients were enrolled onto the trial in 11 European countries and Australia between April 2000 and October 2002. Random assignment to treatment was conducted by block by country. The cutoff point for data reported here is 24 months after enrollment of the last patient.

Patients

Women age 18 to 70 years with HER2-positive MBC were eligible for the trial. Initially, women with IHC 2+ and 3+ disease could be enrolled onto the trial. However, data from other trials indicated that patients with strong HER2 overexpression (IHC 3+) and/or gene amplification (FISH positive) gain the greatest clinical benefit from trastuzumab. This led to a protocol amendment to restrict entry to women with IHC 3+ and/or FISH-positive disease. Patients who had received prior chemotherapy for their metastatic disease or any prior taxanes or anti-HER therapy were excluded. Patients could have received prior (neo) adjuvant anthracyclines (maximum cumulative dose, 360 mg/m^2 doxorubicin or 750 mg/m^2 epirubicin). Baseline left ventricular ejection fraction (LVEF) had to be more than 50%. Hormonal therapy had to be discontinued before the first dose of study drug. Previous radiotherapy was allowed only if treatment had ended at least 14 days before enrollment onto the trial and the patient had fully recovered from all acute adverse effects. Eligible patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2, life expectancy ≥ 12 weeks, and at least one bidimensionally measurable lesion (according to WHO criteria). Bone marrow, renal, and hepatic function had to meet the following criteria: hemoglobin ≥ 10 g/dL and no blood transfusion within the previous 2 weeks; neutrophil count $\geq 2.0 \times 10^9$ cells/L; platelet count $\geq 100 \times 10^9$ cells/L; no evidence of myelodysplastic syndrome or abnormal bone marrow reserve; creatinine $\leq 1.5 \times$ upper limit of normal (ULN) or creatinine clearance ≥ 60 mL/min; total bilirubin less than $1 \times$ ULN; aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) $\leq 2.5 \times$ ULN; and alkaline phosphatase $\leq 5 \times$ ULN (patients with AST and/or ALT $> 1.5 \times$ ULN concomitantly with alkaline phosphatase $> 2.5 \times$ ULN were ineligible for the study). Serum calcium had to be less than 2.8 μM/L/mL at enrollment.

Patients who had brain or leptomeningeal metastases were not eligible for the trial. Patients who had significant cardiac insufficiency (New York Heart Association III or IV), myocardial infarction within the previous 6 months, unstable angina pectoris, uncontrolled arrhythmia, or advanced pulmonary disease or severe dyspnea at rest due to complications of advanced malignancy, or who required supplementary oxygen therapy, were also ineligible for the trial.

Treatment

Docetaxel was to be administered for six cycles at 100 mg/m^2 intravenously every 3 weeks. Trastuzumab was to be administered as a 4 mg/kg intravenous loading dose followed by 2 mg/kg weekly until disease progression. All patients received corticosteroid premedication for the docetaxel infusions, which could include dexamethasone, methylprednisolone, or prednisolone. Patients could receive docetaxel beyond six cycles at the discretion of the investigator. After withdrawal from trial M77001, patients were treated at the discretion of the investigator. Patients experiencing disease progression while receiving docetaxel alone were offered the option to cross over to receive trastuzumab. However, the onset and duration of this therapy was not recorded.

Efficacy Assessments

Tumor response was assessed every third cycle by x-ray, computed tomography scan, magnetic resonance imaging, or clinical examination using the WHO criteria. Patients who did not experience disease progression after 1 year in the main study were observed every 3 weeks until disease progression in an extension phase of the study. For patients with an objective response (complete response [CR] or partial response [PR]) or stable disease, radiologic response was also assessed by an independent radiologic reviewer (IRR). Differences between the IRR and

investigator-assessed best response were reconciled manually by comparison of radiologic assessments and measurements between the IRR and investigator, in which IRR assessments always prevailed. Clinical data were also taken into account (which were not available to the IRR). Subgroup analyses were performed to assess the response rate in relation to IHC 3+ and/or FISH-positive measurable disease, prior adjuvant anthracycline therapy, number of metastatic organ sites, presence of visceral metastases, estrogen receptor (ER) and/or progesterone receptor (PgR) status, age, disease-free interval, and ECOG performance status.

Safety Assessments

Adverse events were assessed every cycle for the duration of the trial and graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC), version 2.0. Data on serious adverse events (SAEs) were collected throughout the study, and data on any drug-related SAEs continued to be collected thereafter. Laboratory assessment of blood counts, clinical chemistry, and liver function was carried out before each cycle and ad hoc as clinically indicated. LVEF was assessed by echocardiography or multiple-gated acquisition scan every third cycle.

Statistical Analyses

The assumption was made that a difference of 15% was to be observed in the ORR between the two arms (from 25% in the docetaxel-alone arm to 40% in the docetaxel plus trastuzumab arm). Using the method of Hauck-Anderson, a sample size of 70 assessable patients per arm would allow this difference to be reported with a precision of ± 16.1%, hence giving a 95% CI for the difference in ORR of −1.1% to +31.1%. To allow for nonassessable patients, the total number of patients required for random assignment to treatment was 186 (93 per arm). For response end points, response rates and 95% CI were calculated, with differences between groups tested at the 5% significance level by a two-sided χ^2 test. Response rate differences in various subgroups were presented in a Forest plot, showing odds ratios and 95% confidence limits for treatment differences in each subgroup. For time to event end points, Kaplan-Meier curves were calculated, with differences between groups tested at the 5% significance level by a two-sided log-rank test. Retrospectively, Kaplan-Meier curves were calculated for the subgroup of patients in the docetaxel-alone arm who crossed over to trastuzumab and those who did not cross over.

Conduct of Study

Written informed consent was obtained from all patients before enrollment and the study was conducted in accordance with International Conference on Harmonisation Good Clinical Practice and the principles of the Declaration of Helsinki on the rights of research participants.

RESULTS

Patient Demographics

One hundred eighty-eight patients were randomly assigned to study medication; 94 patients were assigned in each arm. Two patients (both in the trastuzumab plus docetaxel arm) withdrew before receiving the first dose. Baseline patient characteristics (Table 1) were generally balanced between the two arms, although there were more patients with ER- or PgR-positive disease in the docetaxel-alone arm compared with the combination arm (56% *v* 41%), and more patients had received prior (neo)adjuvant anthracyclines in the combination arm compared with the docetaxel-alone arm (64% *v* 55%). Ninety-five percent of patients had IHC 3+ and/or FISH-positive disease; 87% had IHC 3+ disease. The median duration of primary disease (first diagnosis to diagnosis of metastasis) was 26.6 and 22.6 months, and the median duration of metastatic disease was 1.3 months and 1 month, in the combination arm and the docetaxel-alone arm, respectively. A small number of patients, who had a prolonged duration of metastatic disease before study entry, had received hormonal anticancer therapy in the metastatic setting.

Efficacy

Exposure to docetaxel was similar in the two treatment arms. There was a median number of six treatment cycles in both arms (range, one to 14 in the docetaxel-alone arm and one to 15 in the combination arm). More than six cycles of docetaxel were administered to 36 patients (38%) in the docetaxel-alone arm and 30 patients (33%) in the combination arm. The median cumulative dose of docetaxel was 1,044 mg (range, 47 to 2,280 mg) in the docetaxel-alone arm and 1,018 mg (range, 130 to 2,856 mg) in the combination arm. The median number of trastuzumab infusions was 39 (range, one to 171), with a median cumulative dose of 5,044 mg (range, 204 to 25,308 mg).

In the intent-to-treat (ITT) analysis, the ORR in the combination arm was 61% (95% CI, 50% to 71%), comprising six CRs (7%) and 50 PRs (54%) compared with an ORR of 34% (95% CI, 25% to 45%) in the docetaxel-alone arm, comprising two CRs (2%) and 30 PRs (32%; $P = .0002$; Table 2). Stable disease was seen in 27% (95% CI, 18% to 37%) and 44% (95% CI, 33% to 54%) of patients in the combination and docetaxel-alone arms, respectively. Seven patients in the docetaxel-alone arm withdrew at cycle 1 or 2: four withdrew as a result of adverse events; one was treated at another hospital; one withdrew when she was found to have disease that was IHC 2+ and FISH negative; and one withdrew when it was found that she had had abnormal baseline liver function tests. A sensitivity analysis was carried out, with all seven of these patients classified as responders and again with all seven excluded from the ITT population, and in both scenarios the difference in ORR between the arms remained significant.

In all subgroups analyzed (exposure to [neo]adjuvant anthracyclines, number of metastatic organ sites, presence of lung and liver metastases, hormone-receptor status, age, disease-free interval, and ECOG performance status), the response rate in the combination arm was superior to that in the docetaxel-alone arm (Fig 1).

There was a statistically significant superiority ($P = .0325$) in OS for trastuzumab plus docetaxel compared with docetaxel alone (Fig 2). Median OS, estimated by the Kaplan-Meier method, was 31.2 months for trastuzumab

Table 1. Patient Characteristics at Baseline (n = 186)

Characteristic	Trastuzumab + Docetaxel (n = 92)		Docetaxel Alone (n = 94)	
	No. of Patients	%	No. of Patients	%
Age, years				
Median	53		55	
Range	32-80		24-79	
HER2 status				
IHC 3+	81	88	82	87
FISH positive	11	12	9	10
IHC 3+ and/or FISH positive	89	97	88	94
Other*	3	3	6	6
Hormone receptor status				
ER positive and/or PgR positive	38	41	53	56
ECOG status				
Median	0		0	
Range	0-4		0-2	
Metastases				
No. lesions/patient				
Median	4		4	
Range	1-12		1-12	
No. sites/patient				
Median	2		2	
Range	1-5		1-5	
Duration primary disease, months				
Median	26.6		22.6	
Range	0.3-267.3		0.2-175.2	
Duration metastatic disease, months				
Median	1.3		1.0	
Range	0.1-67.9		0-66.8	
Site				
Lung	37	40	40	43
Liver	45	49	50	54
Bone	31	34	35	38
Soft tissue	44	48	46	50
Other†	55	60	55	59
Prior therapy				
Adjuvant chemotherapy	65	71	64	68
Adjuvant anthracyclines	59	64	52	55
Hormonal therapy	40	44	44	47
Radiotherapy	59	64	62	66

Abbreviations: HER2, human epidermal growth factor receptor 2; FISH, fluorescence in situ hybridization; ER, estrogen receptor; PrG, progesterone receptor; ECOG, Eastern Cooperative Oncology Group; IHC, immunohistochemistry.
*Of the nine patients in Other category (without IHC 3+ and/or FISH-positive disease), three in the combination arm and five in the docetaxel-alone arm had IHC 2+/FISH-negative disease and one patient in the docetaxel-alone arm had IHC 0/1+ disease and unknown FISH status.
†Other sites of disease included lymph nodes.

plus docetaxel compared with 22.7 months for docetaxel alone. All other time-to-event analyses also favored the trastuzumab plus docetaxel arm: DR (median, 11.7 *v* 5.7 months; $P = .009$); TTP (median, 11.7 *v* 6.1 months; $P = .0001$); and TTF (median, 9.8 *v* 5.3 months; $P = .0001$; Table 2). In total, 41% of patients in the combination arm and 37% of patients in the docetaxel-alone arm were still alive at the data cutoff date. Median follow-up was 40.9 and 35.9 months in the combination and docetaxel-alone arms, respectively.

Fifty-three patients (57%) in the docetaxel-alone arm were reported to have crossed over to receive trastuzumab, usually at disease progression (30 patients) but in some cases after discontinuation of docetaxel because of toxicity (10 patients) or other reasons (13 patients). The median estimated OS in patients who received docetaxel only was 16.6 months, and it was 30.3 months for patients who crossed over to receive trastuzumab at any time point after withdrawal from this trial (Fig 3).

Safety

Adverse events. All 186 patients who received at least one cycle of treatment were included in the safety analysis.

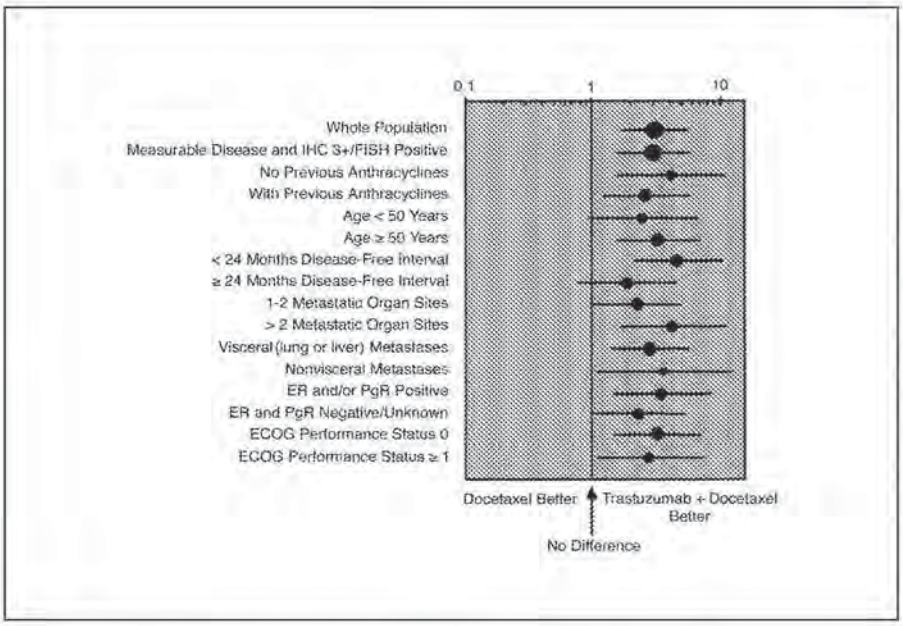

Fig 1. Subgroup analysis: Forest plot of odds ratios ± 95% CIs. IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; ER, estrogen receptor; PgR, progesterone receptor; ECOG, Eastern Cooperative Oncology Group.

The majority of adverse events were mild to moderate in severity. Common nonhematologic events (reported in ≥ 20% of patients, regardless of whether the events were attributable to study treatment) were typical of chemotherapy-related toxicity (eg, alopecia, nausea, and vomiting; Table 3). The incidence of adverse events typically associated with trastuzumab (eg, pyrexia) was seen, as expected, in the combination arm. In addition, there was a slightly higher incidence of typical docetaxel-related adverse events (eg, stomatitis, paraesthesia) in the combination arm and also of events not considered typical for either trastuzumab or docetaxel (eg, headache). Overall, there was a higher incidence of grade 3 (67% *v* 55%) and 4 (34% *v* 23%) adverse events (all causes) in the combination arm compared with the docetaxel-alone arm. However, most of the imbalances noted were due to a higher incidence of grade 1 and 2 toxicities. There were three serious infusion-related reactions: one in the docetaxel-alone arm and two in the combination arm. Of the latter two events, one was related to docetaxel and one to trastuzumab. Overall, fewer patients in the combination arm discontinued treatment due to adverse events compared with those in the docetaxel-alone arm (nine *v* 20 patients, respectively). In the docetaxel-alone arm, 29 patients experienced 42 SAEs; in the combination arm, 38 patients experienced 64 SAEs.

Hematologic toxicity. There was a higher incidence of grade 3/4 leukopenia and neutropenia (20% *v* 15%, and 32% *v* 22%, respectively) in the combination arm compared with the docetaxel-alone arm (Table 4). The incidence of febrile

Table 2. Summary of Efficacy Between the Two Treatment Arms

Outcome	Trastuzumab + Docetaxel (n = 92)	Docetaxel Alone (n = 94)	*P*
ORR, %	61	34	.0002
CR, %	7	2	
PR, %	54	32	
SD, %	27	44	
DR, median, months	11.7	5.7	.009
TTP, median, months	11.7	6.1	.0001
OS, median, months*	31.2	22.7	.0325

Abbreviations: ORR, overall response rate; CR, complete response; PR, partial response; SD, stable disease; DR, duration of response; TTP, time to progression; OS, overall survival.
*Kaplan-Meier estimate.

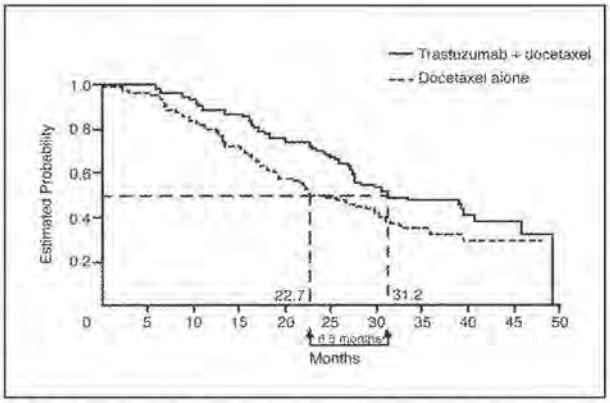

Fig 2. Comparison of estimated overall survival between trastuzumab plus docetaxel and docetaxel-alone arms (Kaplan-Meier plots).

Table 3. Incidence of Nonhematologic Adverse Events (% of patients)

Adverse Event*	Total		Grade 3/4	
	Trastuzumab + Docetaxel (n = 92)	Docetaxel Alone (n = 94)	Trastuzumab + Docetaxel (n = 92)	Docetaxel Alone (n = 94)
Alopecia	67	54	10	6
Asthenia	45	41	10	6
Nausea	45	41	0	1
Diarrhea	43	36	5	2
Peripheral edema	40	35	1	2
Paraesthesia	32	21	0	2
Vomiting	29	22	3	2
Pyrexia	30	15	1	1
Constipation	27	23	2	0
Myalgia	27	26	3	3
Arthralgia	27	20	4	0
Rash	24	12	1	0
Fatigue	24	21	3	3
Mucosal inflammation	23	22	2	4
Erythema	23	11	1	0
Anorexia	22	13	2	0
Headache	21	18	5	1
Increased lacrimation	21	10	1	0
Epistaxis	20	5	0	0

*Reported in ≥ 20% of patients in at least one treatment arm.

neutropenia was 23% in the combination arm (95% CI, 14.7% to 32.8%) compared with 17% in the docetaxel-alone arm (95% CI, 10.0% to 26.2%; Table 4). All patients experiencing febrile neutropenia received empiric antibiotics, with granulocyte colony-stimulating factor administered to four patients in the combination arm and three patients in the docetaxel-alone arm. There were two drug-related deaths as a result of septicemia in the docetaxel arm. All other episodes resolved within 1 week and, in most cases, without recurrence after a dose reduction of docetaxel. Anemia experienced was generally mild. There was an increased incidence in grade 1/2 anemia in the combination arm compared with the docetaxel-alone arm (80% *v* 66%). The incidence of grade 3 anemia was 1% *v* 1%, and no grade 4 anemia was recorded.

Cardiac safety. Slightly more patients experienced decreases in LVEF in the combination arm compared with the docetaxel-alone arm (Table 5): asymptomatic declines in LVEF of 15 or more percentage points occurred in 17% *v* 8% of patients, respectively. Twelve of the 15 patients in the combination arm who experienced a decrease in LVEF had received prior adjuvant anthracycline therapy.

Two patients experienced symptomatic congestive heart failure (CHF); both patients received docetaxel and trastuzumab. These patients had received prior adjuvant anthracycline therapy (cumulative dose 300 mg/m^2 doxorubicin in both patients). The first of these patients entered the trial with a baseline LVEF of 60%. The patient had exertional dyspnea and a decrease in LVEF to 45% during cycle six of treatment and peripheral edema during the following cycle. Two

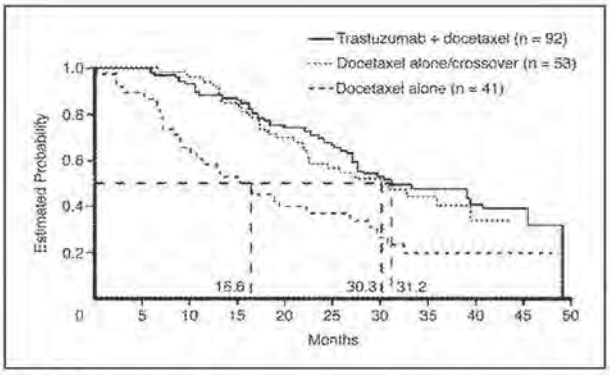

Fig 3. Comparison of estimated overall survival in patients who received trastuzumab and docetaxel first-line treatment versus those who crossed over to receive trastuzumab after progressing on docetaxel alone versus patients who received docetaxel only (Kaplan-Meier plots).

Table 4. Hematologic Toxicity (grade 3/4; % of patients)

Toxicity	Trastuzumab + Docetaxel (n = 92)	Docetaxel Alone (n = 94)
Anemia	1	1
Thrombocytopenia	0	0
Leukopenia	20	15
Neutropenia	32	22
Febrile neutropenia/neutropenic sepsis	23	17

Table 5. Changes in LVEF From Baseline: Worst Value Up to Cycle 6 and Overall (% of patients)

LVEF Worst Value	Up to Cycle 6		Overall*	
	Trastuzumab + Docetaxel (n = 83)	Docetaxel Alone (n = 71)	Trastuzumab + Docetaxel (n = 86)	Docetaxel Alone (n = 76)
Increase or no change	41	41	20	33
Absolute decrease < 15%	48	54	63	60
Absolute decrease ≥ 15%	11	6	17	8
Absolute value < 40%	1	0	1	0

Abbreviation: LVEF, left ventricular ejection fraction.
*LVEF monitoring ceased in patients receiving docetaxel alone after completion of therapy. However, LVEF monitoring continued throughout trastuzumab treatment in the combination arm. The number of LVEF assessments is therefore markedly different in the two arms after cycle 6.

weeks into cycle 7, the dyspnea and peripheral edema were still present and her LVEF had decreased to 40%. The investigator therefore decided to discontinue trastuzumab. Four weeks later, the patient was admitted to the hospital in a coma and died the following day. Death was attributed to progressive metastatic disease. The investigator could not rule out trastuzumab-related cardiotoxicity.

The second patient, who had previously received adjuvant doxorubicin, received six cycles of docetaxel and 97 weekly infusions of trastuzumab, with no evidence of cardiac dysfunction during trastuzumab therapy. One month after discontinuing trastuzumab because of progressive disease, she entered a clinical trial of an investigational anthracycline. Four months later, she experienced fatal biventricular failure, which was considered by the investigator to be related to the novel anthracycline.

DISCUSSION

This randomized, open-label trial compared the efficacy and safety of trastuzumab plus docetaxel versus docetaxel alone in women with HER2-positive MBC. The combination of trastuzumab with docetaxel was superior to docetaxel alone in all clinical efficacy parameters investigated.

Fifty-six patients (61%) achieved a CR or PR in the combination arm compared with 32 patients (34%) in the docetaxel-alone arm ($P = .0002$). The sensitivity analysis showed that the statistically significant difference between the arms remained whether the seven patients in the docetaxel-alone arm not assessable for response (because of early discontinuation of study medication) were counted as responders or excluded from the ITT population. A subgroup analysis of response rates showed a consistent superiority of the combination arm over the docetaxel-alone arm for all baseline characteristics assessed (Fig 1). Apart from more patients with ER- or PgR-positive disease in the docetaxel-alone arm compared with the combination arm (56% *v* 41%) and more patients in the combination arm who had received prior (neo)adjuvant anthracyclines (64% *v* 55%), the arms were well balanced and there is no evidence for any selection bias.

All other efficacy parameters were also improved by the addition of trastuzumab to docetaxel. These included DR (median, 11.7 *v* 5.7 months; $P = .009$), TTP (median, 11.7 *v* 6.1 months; $P = .0001$), and OS (median, 31.2 *v* 22.7 months; $P = .0325$). Importantly, the improvement in survival was seen even though approximately 50% of patients in the docetaxel-alone arm crossed over to receive trastuzumab, which could be expected to dilute any survival benefit conferred by trastuzumab administered as first-line therapy.

Survival was longest for the group who received trastuzumab and docetaxel concomitantly from the start of treatment (median OS, 31.2 months). Patients in the docetaxel-alone arm known to have received trastuzumab after docetaxel appeared to survive longer than those who did not receive subsequent trastuzumab (median OS, 30.3 *v* 16.6 months, respectively). It is possible that earlier treatment with trastuzumab led to the improvement in survival. However, there are possible confounding factors such as poor performance status influencing both the decision to cross the patient over to trastuzumab and the progress of the disease. This analysis was unplanned and explorative, and therefore needs to be interpreted with caution.

The results from this trial of trastuzumab and docetaxel are in line with the findings of the combination pivotal trial (H0648g) of first-line paclitaxel with or without trastuzumab in HER2-positive MBC.[6,7] In the H0648g trial, ORR increased from 17% to 49% with the addition of trastuzumab to paclitaxel in patients with IHC 3+ MBC. In addition, TTP increased from 3.0 to 7.1 months and OS increased from 17.9 to 24.8 months. The improvement in survival was seen even though 72% of patients randomly assigned to paclitaxel alone crossed over to receive trastuzumab at progression. Although there were some differences between the patient populations in the two trials (in particular, all patients in the paclitaxel plus trastuzumab arm of study H0648g had received prior adjuvant anthracycline therapy), the results are nonetheless very similar

between the studies. Most notably, in both trials, patients treated with trastuzumab plus a taxane had a median survival of between 2 and 2.5 years. The concordant results in these two randomized trials provide strong evidence supporting the use of trastuzumab plus a taxane as first-line therapy for women with HER2-positive MBC.

The efficacy gains are significant and achieved without significant increase of toxicity. Overall, the safety profiles of the two arms in trial M77001 were similar. Several of the nonhematologic toxicities commonly seen with docetaxel, such as alopecia and paraesthesia, occurred more frequently in the combination arm, suggesting additivity or even a degree of exacerbation. In the combination arm, there was a greater incidence of infusion-related reactions that can occur with initial doses of trastuzumab. However, it is noteworthy that several of the most troublesome adverse effects associated with chemotherapy, such as nausea, vomiting, and mucositis, were not markedly different between the arms and were generally limited to NCI CTC grades 1 and 2. Grade 3 to 4 toxicities were uncommon and showed little difference between the two groups.

There is an indication of a slight exacerbation by trastuzumab of some docetaxel-related hematologic toxicities, which corroborates a previous report.[22] Episodes of febrile neutropenia were generally manageable, and usually resolved rapidly with standard medical interventions. Furthermore, although there were two deaths as a result of septicemia in the docetaxel-alone arm, none were reported in the combination arm. Although the incidence of adverse events overall was greater in the combination arm, discontinuations because of adverse events were actually more common in the docetaxel-alone arm (9% *v* 20%). This probably reflects the better clinical outcome of patients receiving trastuzumab with docetaxel and the greater willingness of both patients and physicians to persevere in the face of toxicities when disease is improving.

Cardiac dysfunction had been a concern in the H0648g trial. A retrospective analysis showed the incidence of symptomatic heart failure to be approximately 9% among patients receiving trastuzumab plus paclitaxel.[23] Subsequent trials of trastuzumab that have included cardiovascular eligibility criteria and prospective cardiac monitoring have shown an incidence of CHF of less than 4%.[19,24-29] In an analysis of pooled cardiac data from six trastuzumab phase II/III trials (n = 629; 418 with trastuzumab, 211 controls), including trial M77001, the incidence of CHF in trastuzumab-treated patients was only 2.7% (11 of 418).[30] Two of 92 patients (2%) receiving trastuzumab plus docetaxel in trial M77001 developed symptomatic heart failure. Both patients had received prior adjuvant anthracyclines. One patient developed CHF during the study. The other patient developed CHF 5 months after discontinuation of trastuzumab, and had received an investigational anthracycline only a month after completing trastuzumab, despite the recommended 24-week washout period between completing trastuzumab and commencing anthracycline therapy. These data suggest that treatment with trastuzumab in combination with docetaxel has manageable cardiac toxicity, the incidence of which is within the range expected for patients receiving trastuzumab plus chemotherapy in more recent clinical trials.

The results of the M77001 trial, which show a significant survival benefit for trastuzumab in combination with docetaxel, provide for an additional valuable first-line treatment option in routine clinical practice for patients with HER2-positive MBC. Other trials are taking the trastuzumab plus docetaxel combination forward, both in the metastatic and adjuvant settings. Randomized trials in patients with HER2-positive MBC are being performed to investigate the additional benefit of adding in a third drug, such as capecitabine (as in the ongoing MO16419 trial) or carboplatin (as in the BCIRG 007 trial, which has recently completed recruitment). Four large adjuvant trials of trastuzumab in patients with HER2-positive breast cancer are currently being conducted: the Herceptin Adjuvant Trial (HERA), National Surgical Adjuvant Breast and Bowel (NSABP) Project, Intergroup, and Breast Cancer International Research Group (BCIRG) 006 trials. Whereas trastuzumab is administered as monotherapy in the HERA trial after the completion of standard adjuvant chemotherapy, the NSABP and Intergroup trials investigate trastuzumab administered either concurrently or sequentially with paclitaxel, after anthracycline plus cyclophosphamide chemotherapy. In the BCIRG 006 trial, docetaxel is administered either on its own or concurrently with trastuzumab, after AC chemotherapy; a third arm of this trial is examining the combination of docetaxel, trastuzumab, and carboplatin (analogous to BCIRG 007). All of these trials have passed their per-protocol scheduled interim safety analyses. The final results of these adjuvant trials are awaited with great interest.

In the meantime, recent results of a randomized trial of trastuzumab administered as neoadjuvant therapy in patients with HER2-positive operable breast cancer showed that the addition of trastuzumab provided a significant increase in the pathologic CR rate in combination with paclitaxel and fluorouracil, epirubicin, and cyclophosphamide compared with chemotherapy alone.[31]

In summary, the results of the M77001 trial, together with the H0648g trial (trastuzumab in combination with paclitaxel) provide strong evidence that the combination of trastuzumab with a taxane is efficacious and well tolerated as first-line therapy for women with HER2-positive MBC, offering a significant survival benefit as well as a higher rate and longer duration of responses, compared with a taxane alone. In addition, the benefit shown for trastuzumab in this trial supports ongoing investigations of trastuzumab in

the neoadjuvant and adjuvant treatment of patients with HER2-positive breast cancer.

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Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Acted as a consultant within the last 2 years: Michel Marty, IGR; Ray Snyder, Aventis; Kenneth O'Byrne, Roche. Performed contract work within the last 2 years: Michel Marty, IGR; Ray Snyder, Aventis. Served as an officer or member of the Board of a company: Ray Snyder, Aventis. Received more than $2,000 a year from a company for either of the last 2 years: Kenneth O'Byrne, Roche; Carol Ward, Roche; Karen Mayne, Roche.

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Patent Docket P1256R1D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Susan D. Hellmann et al. Serial No.: 10/356,824 Filed: February 3, 2003 For: TREATMENT WITH ANTI-ErbB2 ANTIBODIES	Group Art Unit: 1643 Examiner: Anne Holleran Confirmation No: 4326 CUSTOMER NO: 09157 **Electronically Filed On:** February 8, 2008

PETITION AND FEE FOR TWO MONTH EXTENSION OF TIME (37 CFR 1.136(a))

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

Sir:

Applicants petition the Commissioner of Patents and Trademarks to extend the time for response to the Office Action dated September 11, 2007 for two months from December 11, 2007 to February 11, 2008. The extended time for response does not exceed the statutory period.

Please charge Deposit Account No. 07-0630 in the amount of $460 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

Respectfully submitted,

GENENTECH, INC.

Date: February 8, 2008

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Electronic Patent Application Fee Transmittal

Application Number:	10356824
Filing Date:	03-Feb-2003
Title of Invention:	Treatment with anti-ErbB2 antibodies
First Named Inventor/Applicant Name:	Virginia E. Paton
Filer:	Wendy M. Lee/Anna Kan
Attorney Docket Number:	P1256R1D1

Filed as Large Entity

Utility Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
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Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 2 months with $0 paid	1252	1	460	460

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	460

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Application Number:	10356824
International Application Number:	
Confirmation Number:	4326
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First Named Inventor/Applicant Name:	Virginia E. Paton
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	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Amendment - After Non-Final Rejection		1	1	
	Claims		2	4	
	Applicant Arguments/Remarks Made in an Amendment		5	13	
Warnings:					
Information:					
2	Examination support document	baselga10.pdf	73573 d9c63315e1bd5e607b3ae114c22c06338474ca3f	no	1
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Information:					
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Warnings:					
Information:					
6	Examination support document	marty.pdf	306592 9e534d59d5d3f563d2775ed85c59672899c09ac1	no	10
Warnings:					
Information:					

7	Extension of Time	P1256R1D1ext.pdf	31179 5d773e951992682af4655e85caf9488f1 67352f4	no	1
Warnings:					
Information:					
8	Fee Worksheet (PTO-06)	fee-info.pdf	8133 2bbed1db338e8e3ee4e911c21c9b28b a1428d5	no	2
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Patent Docket P1256R1D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Susan D. Hellmann et al. Serial No.: 10/356,824 Filed: February 3, 2003 For: TREATMENT WITH ANTI-ErbB2 ANTIBODIES	Group Art Unit: 1643 Examiner: Anne Holleran Confirmation No: 4326 CUSTOMER NO: 09157 **Electronically Filed On:** February 8, 2008

INFORMATION DISCLOSURE STATEMENT

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

Sir:

Applicants submit herewith patents, publications or other information (listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement is filed in accordance with the provisions of:

( ) **37 CFR §1.97(b)**
- within three months of the filing date of the application other than a continued prosecution application under 37 CFR §1.53(d); **or**
- within three months of the date of entry of the national stage of a PCT application as set forth in 37 CFR§1.491, **or**
- before the mailing of the first Office action on the merits; **or**
- before the mailing of the first Office action after the filing of a request for a continued examination under 37 CFR §1.114.

(X) **37 CFR §1.97(c)**
- by the applicant after the period specified in 37 CFR §1.97(b), but prior to the mailing date of any of a final action under 37 CFR §1.113, or a notice of allowance under 37 CFR §1.311, or an action that otherwise closes

prosecution in the application, and is accompanied by either the fee set forth in 37 CFR §1.17(p) **or** a statement as specified in 37 CFR §1.97(e), as checked below.

0 **37 CFR §1.97(d)**
- after the period specified in CFR §1.97(c), and is accompanied by the fee set forth in 37 CFR §1.17(p) **and** a statement as specified in 37 CFR §1.97(e), as checked below.

(If either of boxes 37 CFR §1.97(c) or 37 CFR §1.97(d) is checked above, the following statement under 37 CFR §1.97(e) may need to be completed and/or the fee paid.)

0 **37 CFR §1.97(e)**Each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.

(X) The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $180.00 to cover the cost of this Information Disclosure Statement under 37 CFR §1.17(p). Any deficiency or overpayment should be charged or credited to this deposit account.

0 **37 CFR §1.704(d)** Each item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application and the communication was not received by any individual designated in §1.56(c) more than thirty days prior to the filing of this information disclosure statement. Therefore, in accordance with the provisions of 37 CFR §1.704(d), the filing of this information disclosure statement will not be considered a failure to engage in reasonable efforts to conclude prosecution under 37 CFR §1.704.

A list of the patent(s) and/or publication(s) is set forth on the attached revised Form PTO-1449. Copies of the items listed on the PTO-1449 form are supplied herewith, except for (i) United States patent(s) and United States patent application publication(s) and (ii) additional documents that are marked with an asterisk (*) in the attached PTO-1449 form. Copies of United States patents and United States patent application publications will not be supplied unless requested by the Office (37 CFR §1.98(a)(2)(ii)). See Final Rule **1287 OG** (October 12, 2004). Additional documents cited with an asterisk have not been supplied because they were previously cited by or submitted to the Office in prior application Serial No. 09/208,649, filed December 10, 1998 and benefit from the prior application is claimed in this application under 35 U.S.C §120. However, copies of any cited document will be provided in its entirety at the request of the Office.

0 BLAST results enclosed:

The undersigned also wishes to bring to the attention of the Examiner BLAST results of computerized alignments of the against sequences contained in the nucleotide and protein databases. The BLAST results are provided in paper form and are identified as reference "BLAST Results A-1- A-0" (nucleotide) and "BLAST Results B-1 - B-0" (protein) on the PTO Form 1449. Applicant requests that these references also be considered and that the Form 1449 be initialed to indicate the Examiner's consideration of the references.

A concise explanation of relevance of the items listed on PTO-1449 is:

(X) not given

0 given for each listed item

0 given for non-English language listed item(s) (Required)

0 in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

In accordance with 37 CFR §1.97(g), the filing of this information disclosure statement shall not be construed as a representation that a search has been made.

In accordance with 37 CFR §1.97(h), the filing of this information disclosure statement shall not be construed to be an admission that the information cited in the statement is, or is considered to be, material to patentability as defined in 37 CFR § 1.56(b).

The Commissioner is hereby authorized to charge any additional fees required under 37 CFR 1.16 and 1.17 for this Information Disclosure Statement, or credit overpayment to Deposit Account No. 07-0630.

Respectfully submitted,

GENENTECH, INC.

Date: February 8, 2008

By: /Wendy M. Lee/
Wendy M. Lee
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Telephone No. (650) 225-1994

FORM PTO-1449 U.S. Dept. of Commerce Patent and Trademark Office

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P1256R1D1	10/356,824
Applicant: Hellmann et al.	
Filing Date: 03 Feb 2003	Group: 1643

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
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Examiner | Date Considered

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FORM PTO-1449	U.S. Dept. of Commerce Patent and Trademark Office	Atty Docket No. P1256R1D1	Serial No. 10/356,824
LIST OF DISCLOSURES CITED BY APPLICANT (Use several sheets if necessary)		Applicant Hellmann et al.	
		Filing Date 03 Feb 2003	Group 1643

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Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
	317	WO 00/69460 A1	23.11.00	PCT				
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(54) Title: TREATMENT WITH ANTI–ErbB2 ANTIBODIES

(57) Abstract

A method treating a human patient to or diagnosed with a tumor in which ErbB2 protein is expressed comprising the following steps, performed sequentially: (a) treating the patient with a therapeutically effective amount of an anti–ErbB2 antibody; (b) surgically removing the tumor, and then (c) treating the patient with a therapeutically effective amount of an anti–ErbB2 antibody or of a chemotherapeutic agent.

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TREATMENT WITH ANTI-ErbB2 ANTIBODIES

Field of the Invention

The present invention concerns the treatment of cancer with anti-ErbB2 antibodies.

Background of the Invention

Proto-oncogenes that encode growth factors and growth factor receptors have been identified to play important roles in the pathogenesis of various human malignancies, including breast cancer. It has been found that the human *erb*B2 gene (also known as *HER2*, or *c-erbB-2*), which encodes a 185-kd transmembrane glycoprotein receptor ($p185^{HER2}$) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer (Slamon *et al.*, *Science* 235:177-182 [1987]; Slamon *et al.*, *Science* 244:707-712 [1989]).

Several lines of evidence support a direct role for ErbB2 in the pathogenesis and clinical aggressiveness of ErbB2-overexpressing tumors. The introduction of ErbB2 into non-neoplastic cells has been shown to cause their malignant transformation (Hudziak *et al.*, *Proc. Natl. Acad. Sci. USA* 84:7159-7163 [1987]; DiFiore *et al.*, *Science* 237:78-182 [1987]). Transgenic mice that express HER2 were found to develop mammary tumors (Guy *et al.*, *Proc. Natl. Acad. Sci. USA* 89:10578-10582 [1992]).

Antibodies directed against human ErbB2 protein and the protein encoded by the rat equivalent of the *erb*B2 gene (*neu*) have been described. Drebin *et al.*, *Cell* 41:695-706 (1985) refer to an IgG2a monoclonal antibody which is directed against the rat *neu* gene product. This antibody called 7.16.4 causes down-modulation of cell surface p185 expression on B104-1-1 cells (NIH-3T3 cells transfected with the *neu* protooncogene) and inhibits colony formation of these cells. In Drebin *et al. PNAS (USA)* 83:9129-9133 (1986), the 7.16.4 antibody was shown to inhibit the tumorigenic growth of *neu*-transformed NIH-3T3 cells as well as rat neuroblastoma cells (from which the *neu* oncogene was initially isolated) implanted into nude mice. Drebin *et al.* in *Oncogene* 2:387-394 (1988) discuss the production of a panel of antibodies against the rat *neu* gene product. All of the antibodies were found to exert a cytostatic effect on the growth of *neu*-transformed cells suspended in soft agar. Antibodies of the IgM, IgG2a and IgG2b isotypes were able to mediate significant *in vitro* lysis of *neu*-transformed cells in the presence of complement, whereas none of the antibodies were able to mediate high levels of antibody dependent cellular cytotoxicity (ADCC) of the *neu*-transformed cells. Drebin *et al. Oncogene* 2:273-277 (1988) report that mixtures of antibodies reactive with two distinct regions on the p185 molecule result in synergistic anti-tumor effects on *neu*-transformed NIH-3T3 cells implanted into nude mice. Biological effects of anti-*neu* antibodies are reviewed in Myers *et al.*, *Meth. Enzym.* 198:277-290 (1991). See also WO 94/22478 published October 13, 1994.

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 SKBR3. Relative cell proliferation of the SKBR3 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, including 7C2 and 7F3, reduced cellular proliferation to a lesser extent in this assay. Hudziak *et al.* conclude that the effect of the 4D5 antibody on SKBR3 cells was cytostatic rather than cytotoxic, since SKBR3 cells resumed growth at a nearly normal rate following removal of

the antibody from the medium. The antibody 4D5 was further found to sensitize $p185^{erbB2}$-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also WO89/06692 published July 27, 1989. 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); and D'souza *et al. Proc. Natl. Acad. Sci.* 91:7202-7206 (1994).

Tagliabue *et al. Int. J. Cancer* 47:933-937 (1991) describe two antibodies which were selected for their reactivity on the lung adenocarcinoma cell line (Calu-3) which overexpresses ErbB2. One of the antibodies, called MGR3, was found to internalize, induce phosphorylation of ErbB2, and inhibit tumor cell growth *in vitro*.

McKenzie *et al. Oncogene* 4:543-548 (1989) generated a panel of anti-ErbB2 antibodies with varying epitope specificities, including the antibody designated TA1. This TA1 antibody was found to induce accelerated endocytosis of ErbB2 (see Maier *et al. Cancer Res.* 51:5361-5369 (1991)). Bacus *et al. Molecular Carcinogenesis* 3:350-362 (1990) reported that the TA1 antibody induced maturation of the breast cancer cell lines AU-565 (which overexpresses the *erb*B2 gene) and MCF-7 (which does not). Inhibition of growth and acquisition of a mature phenotype in these cells was found to be associated with reduced levels of ErbB2 receptor at the cell surface and transient increased levels in the cytoplasm.

Stancovski *et al. PNAS (USA)* 88:8691-8695 (1991) generated a panel of anti-ErbB2 antibodies, injected them i.p. into nude mice and evaluated their effect on tumor growth of murine fibroblasts transformed by overexpression of the *erb*B2 gene. Various levels of tumor inhibition were detected for four of the antibodies, but one of the antibodies (N28) consistently stimulated tumor growth. Monoclonal antibody N28 induced significant phosphorylation of the ErbB2 receptor, whereas the other four antibodies generally displayed low or no phosphorylation-inducing activity. The effect of the anti-ErbB2 antibodies on proliferation of SKBR3 cells was also assessed. In this SKBR3 cell proliferation assay, two of the antibodies (N12 and N29) caused a reduction in cell proliferation relative to control. The ability of the various antibodies to induce cell lysis *in vitro* via complement-dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC) was assessed, with the authors of this paper concluding that the inhibitory function of the antibodies was not attributed significantly to CDC or ADCC.

Bacus *et al. Cancer Research* 52:2580-2589 (1992) further characterized the antibodies described in Bacus *et al.* (1990) and Stancovski *et al.* of the preceding paragraphs. Extending the i.p. studies of Stancovski *et al.*, the effect of the antibodies after i.v. injection into nude mice harboring mouse fibroblasts overexpressing human ErbB2 was assessed. As observed in their earlier work, N28 accelerated tumor growth whereas N12 and N29 significantly inhibited growth of the ErbB2-expressing cells. Partial tumor inhibition was also observed with the N24 antibody. Bacus *et al.* also tested the ability of the antibodies to promote a mature phenotype in the human breast cancer cell lines AU-565 and MDA-MB453 (which overexpress ErbB2) as well as MCF-7 (containing low levels of the receptor). Bacus *et al.* saw a correlation between tumor inhibition *in vivo* and cellular differentiation;

the tumor-stimulatory antibody N28 had no effect on differentiation, and the tumor inhibitory action of the N12, N29 and N24 antibodies correlated with the extent of differentiation they induced.

Xu *et al. Int. J. Cancer* 53:401-408 (1993) evaluated a panel of anti-ErbB2 antibodies for their epitope binding specificities, as well as their ability to inhibit anchorage-independent and anchorage-dependent growth of SKBR3 cells (by individual antibodies and in combinations), modulate cell-surface ErbB2, and inhibit ligand stimulated anchorage-independent growth. See also WO94/00136 published Jan 6, 1994 and Kasprzyk *et al. Cancer Research* 52:2771-2776 (1992) concerning anti-ErbB2 antibody combinations. Other anti-ErbB2 antibodies are discussed in 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); and Harwerth *et al. J. Biol. Chem.* 267:15160-15167 (1992).

A recombinant humanized anti-ErbB2 monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2 or HERCEPTIN®) has been clinically active in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior anticancer therapy. (Baselga *et al., J. Clin. Oncol.* 14:737-744 [1996]).

ErbB2 overexpression is commonly regarded as a predictor of a poor prognosis, especially in patients with primary disease that involves axillary lymph nodes (Slamon *et al.*, [1987] and [1989], *supra*; Ravdin and Chamness, *Gene* 159:19-27 [1995]; and Hynes and Stern, *Biochim Biophys Acta* 1198:165-184 [1994]), and has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluoruracil) and anthracyclines (Baselga *et al.*, *Oncology* 11(3 Suppl 1):43-48 [1997]). However, despite the association of ErbB2 overexpression with poor prognosis, the odds of HER2-positive patients responding clinically to treatment with taxanes were greater than three times those of HER2-negative patients (*Ibid*). rhuMab HER2 was shown to enhance the activity of paclitaxel (TAXOL®) and doxorubicin against breast cancer xenografts in nude mice injected with BT-474 human breast adenocarcinoma cells, which express high levels of HER2 (Baselga *et al.*, *Breast Cancer, Proceedings of ASCO*, Vol. 13, Abstract 53 [1994]).

Summary of the Invention

In a first aspect, the present invention provides a method of treating a human patient susceptible to or diagnosed with a tumor in which ErbB2 protein is expressed comprising the following steps, performed sequentially:

(a) treating the patient with a therapeutically effective amount of an anti-ErbB2 antibody and, optionally, further comprising treating the patient with a therapeutically effective amount of a chemotherapeutic agent (*e.g.* a taxoid, such as paclitaxel or doxetaxel);

(b) surgically removing the tumor; and then

(c) treating the patient with a therapeutically effective amount of an anti-ErbB2 antibody and/or of a chemotherapeutic agent (*e.g.* a taxoid, such as paclitaxel or doxetaxel).

Preferably, the tumor overexpresses ErbB2 protein and is selected from the group consisting of a breast tumor, squamous cell tumor, small-cell lung tumor, non-small cell lung tumor, gastrointestinal tumor, pancreatic tumor, glioblastoma, cervical tumor, ovarian tumor, liver tumor, bladder tumor, hepatoma, colon tumor, colorectal

tumor, endometrial tumor, salivary gland tumor, kidney tumor, prostate tumor, vulval tumor, thyroid tumor, hepatic tumor, head tumor and neck tumor.

The invention further provides an article of manufacture comprising a container, a composition within the container comprising an anti-ErbB2 antibody and a package insert instructing the user of the composition to treat a patient essentially according to the above method.

Brief Description of the Drawings

Fig. 1 shows epitope-mapping of the extracellular domain of ErbB2 as determined by truncation mutant analysis and site-directed mutagenesis (Nakamura *et al. J. of Virology* 67(10):6179-6191 [Oct 1993]; Renz *et al. J. Cell Biol.* 125(6):1395-1406 [Jun 1994]). The anti-proliferative MAbs 4D5 and 3H4 bind adjacent to the transmembrane domain. The various ErbB2-ECD truncations or point mutations were prepared from cDNA using polymerase chain reaction technology. The ErbB2 mutants were expressed as gD fusion proteins in a mammalian expression plasmid. This expression plasmid uses the cytomegalovirus promoter/enhancer with SV40 termination and polyadenylation signals located downstream of the inserted cDNA. Plasmid DNA was transfected into 293S cells. One day following transfection, the cells were metabolically labeled overnight in methionine and cysteine-free, low glucose DMEM containing 1% dialyzed fetal bovine serum and 25 µCi each of ^{35}S methionine and ^{35}S cysteine. Supernatants were harvested either the ErbB2 MAbs or control antibodies were added to the supernatant and incubated 2-4 hours at 4°C. The complexes were precipitated, applied to a 10-20% Tricine SDS gradient gel and electrophoresed at 100 V. The gel was electroblotted onto a membrane and analyzed by autoradiography. SEQ ID NOs:3 and 4 depict the 3H4 and 4D5 epitopes, respectively.

Fig. 2 depicts with underlining the amino acid sequence of Domain 1 of ErbB2 (SEQ ID NO:1). Bold amino acids indicate the location of the epitope recognized by MAbs 7C2 and 7F3 as determined by deletion mapping, *i.e.* the "7C2/7F3 epitope" (SEQ ID NO:2).

Detailed Description of the Preferred Embodiments

I. Definitions

The terms "HER2", "ErbB2" and "c-Erb-B2" are used interchangeably. Unless indicated otherwise, the terms "ErbB2", "c-Erb-B2" and "HER2" refer to the human protein, and "*Her2*", "*erb*B2" and "*c-erb-B2*" refer to human gene. The human *erb*B2 gene and ErbB2 protein are, for example, described in Semba *et al.*, *PNAS (USA)* 82:6497-6501 (1985) and Yamamoto *et al. Nature* 319:230-234 (1986) (Genebank accession number X03363). ErbB2 comprises four domains (Domains 1-4).

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 region of ErbB2. To screen for antibodies which bind to the 4D5 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 (see Fig. 1) to assess whether the antibody binds to the 4D5 epitope of ErbB2 (*i.e.* any one or more residues in the region from about residue 529, *e.g.* about residue 561 to about residue 625, inclusive; SEQ ID NO:4).

The "epitope 3H4" is the region in the extracellular domain of ErbB2 to which the antibody 3H4 binds. This epitope is shown in Fig. 1, and includes residues from about 541 to about 599, inclusive, in the amino acid sequence of ErbB2 extracellular domain (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 (*i.e.* any one or more of residues in the region from about residue 22 to about residue 53 of ErbB2 [SEQ ID NO:2]).

The term "induces cell death" or "capable of inducing cell death" refers to the ability of the antibody to make a viable cell become nonviable. The "cell" here is one which expresses the ErbB2 receptor, especially where the cell overexpresses the ErbB2 receptor. A cell which "overexpresses" ErbB2 has significantly higher than normal ErbB2 levels compared to a noncancerous cell of the same tissue type. Preferably, the cell is a cancer cell, *e.g.* a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. *In vitro*, the cell may be a SKBR3, 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 cellular 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 antibody 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".

The phrase "induces apoptosis" or "capable of inducing apoptosis" refers to the ability of the antibody to induce programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmatic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is 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 or bladder cell. *In vitro*, the cell may be a SKBR3, 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 as disclosed in the example herein; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody 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" (see below).

Sometimes the pro-apoptotic antibody will be one which blocks HRG binding/activation of the ErbB2/ErbB3 complex (*e.g.* 7F3 antibody). In other situations, the antibody is one which does not significantly block activation of the ErbB2/ErbB3 receptor complex by HRG (*e.g.* 7C2). Further, the antibody may be one like

7C2 which, while inducing apoptosis, does not induce 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).

The antibody of interest may be one like 7C2 which binds specifically to human ErbB2 and does not significantly cross-react with other proteins such as those encoded by the *erb*B1, *erb*B3 and/or *erb*B4 genes. Sometimes, the antibody may 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). In such embodiments, the extent of binding of the antibody to these proteins (*e.g.*, cell surface binding to endogenous receptor) will be less than about 10% as determined by fluorescence activated cell sorting (FACS) analysis or radioimmunoprecipitation (RIA).

"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.* $HRG\beta1_{177-244}$).

The "ErbB2-ErbB3 protein complex" and "ErbB2-ErbB4 protein complex" are noncovalently associated oligomers of the ErbB2 receptor and the ErbB3 receptor or ErbB4 receptor, respectively. The complexes form when a cell expressing both of these receptors is exposed to HRG 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).

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

"Native antibodies" and "native immunoglobulins" 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- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the 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 complementarity determining regions (CDRs) or 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 region (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and

in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *NIH Publ. No.91-3242*, Vol. I, pages 647-669 [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 toxicity.

Papain digestion of antibodies produces two identical antigen- binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: 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 immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antibody" 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.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata *et al. Protein Eng.* 8(10):1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

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 conventional (polyclonal) antibody preparations which typically 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 are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. 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. Patent 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 (immunoglobulins) 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. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize 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 CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally 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); Reichmann *et al.*, *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992). The humanized antibody includes a PRIMATIZED™ antibody wherein the antigen-binding

region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

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.

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.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal is human.

A "disorder" is any condition that would benefit from treatment with the anti-ErbB2 antibody. 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; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

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 disorder. 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 tumor progression (TTP), determining the response rate (RR) and/or evaluating overall survival.

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, 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, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma as well as head and neck cancer.

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.* I^{131}, I^{123}, I^{125}, Y^{90}, At^{211}, Cu^{67}, Bi^{212}, Pd^{109}, Re^{188} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments 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, carzinophilin, chromomycins, dactinomycin, daunorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, 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; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.* paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, NJ) 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; carminomycin; 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 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 anti-androgens such as flutamide and nilutamide; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially an ErbB2-overexpressing cancer cell either *in vitro* or *in vivo*. Thus, the growth inhibitory agent is one which significantly reduces the percentage of ErbB2 overexpressing 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), taxol, 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, oncogens, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13. The 4D5 antibody (and functional equivalents thereof) can also be employed for this purpose.

"Doxorubicin" is an athracycline antibiotic. The full chemical name of doxorubicin is (8S-cis)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexapyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve

growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

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.

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 anthracycline antibiotic and/or an anti-ErbB2 antibody, 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 MR, 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.

II. Production of anti-ErbB2 antibodies

A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention. 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 SKBR3 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 1/5 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. Patent 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, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland 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); 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 immunoglobulin 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 immunoglobulin 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, 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- and light-chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; 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 and human antibodies

Methods for humanizing non-human antibodies are well known 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 rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent 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 CDR 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 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 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. Immnol.*, 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 CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, 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). Human antibodies can also be derived from phage-display libraries (Hoogenboom *et al., J. Mol. Biol.*, 227:381 (1991); Marks *et al., J. Mol. Biol.*, 222:581-597 (1991)).

(iv) 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.

(v) 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. For example, one arm may bind an epitope in Domain 1 of ErbB2 such as the 7C2/7F3 epitope, the other may bind a different ErbB2 epitope, *e.g.* the 4D5 epitope. 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. These antibodies possess an ErbB2-binding arm and an arm which binds the cytotoxic agent (*e.g.* saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments [*e.g.* $F(ab')_2$ bispecific antibodies].

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 W096/27011, 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.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

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).

(vi) Screening for antibodies with the desired properties

Techniques for generating antibodies have been described above. Those antibodies having the characteristics described herein are selected.

To select for antibodies which induce cell death, loss of membrane integrity as indicated by, *e.g.*, PI, trypan blue or 7AAD uptake is 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 x 20 mm dishes and allowed to attach overnight. The medium is then removed and replaced with fresh medium alone or medium containing 10µg/ml of the appropriate MAb. 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 1200rpm 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 x 75 tubes (1ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10µg/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 are selected.

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 10µg/ml of the MAb. 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 labelled annexin (*e.g.* annexin V-FTIC) (1µg/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 9µg/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 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, epitope mapping can be performed by methods known in the art.

To identify anti-ErbB2 antibodies which inhibit growth of SKBR3 cells in cell culture by 50-100%, the SKBR3 assay described in WO89/06692 can be performed. According to this assay, SKBR3 cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillinstreptomycin. The SKBR3 cells are plated at 20,000 cells in a 35mm cell culture dish (2mls/35mm dish). 2.5μg/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 SKBR3 cells by 50-100% are selected for combination with the apoptotic antibodies as desired.

(vii) Effector function engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody in treating cancer, for example. For example 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).

(viii) Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* a small molecule toxin or an enzymatically active toxin of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above.

Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, a maytansine (U.S. Patent No. 5,208,020), a trichothene, and CC1065 are also contemplated herein. In one preferred embodiment of the invention, the antibody is conjugated to one or more maytansine molecules (*e.g.* about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antibody (Chari *et al. Cancer Research* 52: 127-131 [1992]) to generate a maytansinoid-antibody immunoconjugate.

Another immunoconjugate of interest comprises an anti-ErbB2 antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman *et al. Cancer Research* 53: 3336-3342 [1993] and Lode *et al. Cancer Research* 58: 2925-2928 [1998]).

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana*

proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

The present invention further contemplates an immunoconjugate formed between an antibody and a compound with nucleolytic activity (*e.g.* a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

A variety of radioactive isotopes are available for the production of radioconjugated anti-ErbB2 antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) 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 tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari *et al. Cancer Research* 52: 127-131 [1992]) may be used.

Alternatively, a fusion protein comprising the anti-ErbB2 antibody and cytotoxic agent may be made, *e.g.* by recombinant techniques or peptide synthesis.

In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.* avidin) which is conjugated to a cytotoxic agent (*e.g.* a radionucleotide).

(ix) Immunoliposomes

The anti-ErbB2 antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al., Proc. Natl Acad. Sci. USA*, 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent or DNA (e.g.

for gene therapy) is optionally contained within the liposome. See Gabizon *et al. J. National Cancer Inst.* 81(19)1484 (1989)

(x) Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT)

The antibodies of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 [1987]). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the anti-ErbB2 antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.*, Neuberger *et al.*, *Nature*, 312: 604-608 [1984]).

(xi) Antibody-salvage receptor binding epitope fusions

In certain embodiments of the invention, it may be desirable to use an antibody fragment, rather than an intact antibody, to increase tumor penetration, for example. In this case, it may be desirable to modify the antibody fragment in order to increase its serum half life. This may be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment (*e.g.* by mutation of the appropriate region in the antibody fragment or by incorporating the epitope into a peptide tag that is then fused to the antibody fragment at either end or in the middle, *e.g.*, by DNA or peptide synthesis).

A systematic method for preparing such an antibody variant having an increased *in vivo* half-life comprises several steps. The first involves identifying the sequence and conformation of a salvage receptor binding epitope of an Fc region of an IgG molecule. Once this epitope is identified, the sequence of the antibody of interest is modified to include the sequence and conformation of the identified binding epitope. After the sequence is mutated, the antibody variant is tested to see if it has a longer *in vivo* half-life than that of the original

antibody. If the antibody variant does not have a longer *in vivo* half-life upon testing, its sequence is further altered to include the sequence and conformation of the identified binding epitope. The altered antibody is tested for longer *in vivo* half-life, and this process is continued until a molecule is obtained that exhibits a longer *in vivo* half-life.

The salvage receptor binding epitope being thus incorporated into the antibody of interest is any suitable such epitope as defined above, and its nature will depend, *e.g.*, on the type of antibody being modified. The transfer is made such that the antibody of interest still possesses the biological activities described herein.

The epitope preferably constitutes a region wherein any one or more amino acid residues from one or two loops of a Fc domain are transferred to an analogous position of the antibody fragment. Even more preferably, three or more residues from one or two loops of the Fc domain are transferred. Still more preferred, the epitope is taken from the CH2 domain of the Fc region (*e.g.*, of an IgG) and transferred to the CH1, CH3, or V_H region, or more than one such region, of the antibody. Alternatively, the epitope is taken from the CH2 domain of the Fc region and transferred to the C_L region or V_L region, or both, of the antibody fragment. See, US Patent 5,739,277 issued April 14, 1998, expressly incorporated herein by reference.

III. Pharmaceutical Formulations

Therapeutic formulations of the antibodies 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).

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 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 comprise a cytotoxic agent, cytokine or growth inhibitory agent. 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].

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

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. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

The anti-ErbB2 antibody conjugated to a biodegradable nanoparticle (*e.g.* polylactic-co-glycolic acid) to increase tumor-specificity is also contemplated herein.

IV. Treatment with the Anti-ErbB2 Antibodies

The invention herein provides a three-step method for treating a human patient susceptible to or diagnosed with a tumor (or tumors) in which ErbB2 protein is expressed. Generally, the tumor to be treated is a primary tumor. In the first step, a therapeutically effective amount of an anti-ErbB2 antibody is administered to the patient in order to reduce the size of, or eliminate, the tumor (or tumors) in the patient prior to surgery. The patient is optionally further treated with one or more chemotherapeutic agents prior to surgery. In the second step, the tumor is surgically removed according to standard surgical procedures (*e.g.* lumpectomy or mastectomy). Following surgery, in the third step, a therapeutically effective amount of an anti-ErbB2 antibody, or of at least one chemotherapeutic agent, is administered to the patient in order to reduce the likelihood of disease recurrence. Generally, an anti-ErbB2 antibody will be administered to the patient following surgery and, optionally, one or more chemotherapeutic agents will further be administered to the patient during this phase of the therapy.

It is contemplated that, according to the present invention, the anti-ErbB2 antibodies may be used to treat a tumor that expresses, and preferably overexpresses, ErbB2 protein. Exemplary conditions or disorders to be treated herein include benign or malignant tumors (*e.g.* renal, liver, kidney, bladder, breast, gastric, ovarian, colorectal, prostate, pancreatic, lung, vulval, thyroid, hepatic carcinomas; sarcomas; glioblastomas; and various head and neck tumors); leukemias and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal,

hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The antibodies of the invention are administered to a human patient, in accord with known methods, such as intravenous administration 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 administration of the antibody is preferred.

Where the anti-ErbB2 antibody is combined with a chemotherapeutic agent, the chemotherapeutic agent is preferably a taxoid, *e.g.* paclitaxel or doxetaxel. Combined administration herein 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. 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). Administration of the chemotherapeutic agent may precede, or follow, administration of the antibody or may be given simultaneously therewith. The antibody may be combined with an anti-estrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) in dosages known for such molecules.

It may be desirable to also administer antibodies against other tumor associated antigens, such as antibodies which bind to the EGFR, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or additionally, two or more anti-ErbB2 antibodies may be co-administered to the patient. Sometimes, it may be beneficial to also administer one or more cytokines to the patient. In a preferred embodiment, the ErbB2 antibody is co-administered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by the ErbB2 antibody. However, simultaneous administration or administration of the ErbB2 antibody first is also contemplated. Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and anti-ErbB2 antibody. Where cardiotoxicity is observed, a cardioprotectant may be administered to the patient as appropriate.

For the prevention or treatment of disease, the appropriate dosage of antibody 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 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.* 0.1-20mg/kg) of antibody 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. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

V. 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. 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. The label or package insert on, or associated with, the container indicates that the composition is used for treating the condition of choice and further indicates treatment of the patient according to the protocol described herein. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as 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.

Deposit of Materials

The following hybridoma cell lines have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 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

Further details of the invention are illustrated by the following non-limiting Example. The disclosures of all citations in the specification are expressly incorporated herein by reference.

Example 1

The anti-ErbB2 $IgG_1\kappa$ murine monoclonal antibody 4D5, specific for the extracellular domain of ErbB2, was produced as described in Fendly *et al.*, *Cancer Research* 50:1550-1558 (1990) and US Patent 5,677,171 issued October 14, 1997. Briefly, NIH 3T3/HER2-3_{400} cells (expressing approximately 1 x 10^5 ErbB2 molecules/cell) produced as described in Hudziak *et al. Proc. Natl. Acad. Sci. (USA)* 84:7159 (1987) were harvested with phospate buffered saline (PBS) containing 25mM EDTA and used to immunize BALB/c mice. The mice were given injections i.p. of 10^7 cells in 0.5ml 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 0.1ml 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. MOPC-21 (IgG1), (Cappell, Durham, NC), was used as an isotype-matched control.

A humanized version of the murine 4D5 antibody (HERCEPTIN®) was engineered by inserting the complementarity determining regions of the murine 4D5 antibody into the framework of a consensus human immunoglobulin (IgG_1) (Carter *et al., Proc. Natl. Acad. Sci. USA* 89:4285-4289 [1992]; and US Patent No.

5,821,337 issued October 13, 1998). The resulting humanized anti-ErbB2 monoclonal antibody has high affinity for $p185^{HER2}$ (Dillohiation constant [K_d]=0.1 nmol/L), markedly inhibits, *in vitro* and in human xenografts, the growth of breast cancer cells that contain high levels of ErbB2, induces antibody dependent cellular cytotoxicity (ADCC), and has been found clinically active, as a single agent, in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior therapy.

HERCEPTIN® is produced by a genetically engineered Chinese hamster ovary (CHO) cell line, grown in large scale, that secretes the antibody into the culture medium. The antibody is purified from the CHO culture media using chromatographic and filtration methods. Each lot of antibody used is assayed to verify identity, purity, and potency, as well as to meet Food and Drug Administration requirements for sterility and safety.

Patients with primary breast tumor presentation characterized by overexpression of the ErbB2 (HER2) oncogene [2+ to 3+ as determined by immunohistochemistry or fluorescence *in situ* hybridization (FISH)] are treated herein. Tumor expression of ErbB2 can be determined by immunohistochemical analysis, as previously described (Slamon *et al., Science* 235:177-182 [1987]; Slamon *et al., Science* 244:707-712 [1989]), of a set of thin sections prepared from the patient's paraffin-archived tumor blocks. Tumors are considered to overexpress ErbB2 if at least 25% of tumor cells exhibit characteristic membrane staining for ErbB2.

Patients are first treated with HERCEPTIN® for 8-24 weeks, optionally in combination with paclitaxel (TAXOL®), in order to reduce the size of, or eliminate, the tumor prior to surgery. On day 0, a 4 mg/kg dose of HERCEPTIN® is administered intravenously, over a 90-minute period. Beginning on day 7, patients receive weekly administration of 2 mg/kg antibody (i.v.) over a 90-minute period. Patients may further receive paclitaxel (TAXOL®). The initial dose of the HERCEPTIN® antibody precedes the first cycle of the chemotherapy regimen by 24 hours. Subsequent doses of the antibody are given immediately before chemotherapy administration, if the initial dose of the antibody is well tolerated. If the first dose of the antibody is not well tolerated, subsequent infusions continue to precede chemotherapy administration by 24 hours. Paciltaxel (TAXOL®) is given at a dose of 175 mg/m^2 over 3 hours by intravenous administration. All patients receiving paclitaxel are premedicated with dexamethasone (or its equivalent) 20 mg x 2, administered orally 12 and 6 hours prior to paclitaxel; diphenhydramine (or its equivalent) 50 mg, iv, administered 30 minutes prior to paclitaxel; and dimetidine (or another H_2 blocker) 300 mg, iv, administered 30 minutes prior to paclitaxel. After the above therapy, classical measures of response may be evaluated immediately prior to surgery; *i.e.*, the sum of the products of the cross-dimensional diameter of any tumor nodules under observation.

Following therapy as described above, the tumor is surgically removed according to standard surgical procedures; lumpectomy or mastectomy. Pathological response may be evaluated at this stage.

After surgery, the patient is treated with HERCEPTIN®, optionally in combination with paclitaxel (TAXOL®), in order to reduce the likelihood of disease recurrence. On day 0, a 4 mg/kg dose of HERCEPTIN® is administered intravenously, over a 90-minute period. Beginning on day 7, patients receive weekly administration of 2 mg/kg antibody (i.v.) over a 90-minute period. Therapy with HERCEPTIN® is continued for one year. Patients may further receive paclitaxel (TAXOL®) for 6-24 weeks. The initial dose of the HERCEPTIN® antibody precedes the first cycle of the chemotherapy regimen by 24 hours. Subsequent doses of the antibody are given

immediately before chemotherapy administration, if the initial dose of the antibody is well tolerated. If the first dose of the antibody is not well tolerated, subsequent infusions continue to precede chemotherapy administration by 24 hours. Paclitaxel (TAXOL®) is given at a dose of 175 mg/m^2 over 3 hours by intravenous administration. All patients receiving paclitaxel are premedicated as described above.

Patients treated according to the above therapeutic regimen will display improved overall survival and/or reduced time to tumor progression (TTP).

Claims:

1. A method of treating a human patient susceptible to or diagnosed with a tumor in which ErbB2 protein is expressed comprising the following steps, performed sequentially:

(a) treating the patient with a therapeutically effective amount of an anti-ErbB2 antibody;

(b) surgically removing the tumor; and then

(c) treating the patient with a therapeutically effective amount of an anti-ErbB2 antibody or of a chemotherapeutic agent.

2. The method of claim 1 wherein step (a) further comprises treating the patient with a therapeutically effective amount of a chemotherapeutic agent.

3. The method of claim 1 wherein step (c) comprises treating the patient with a therapeutically effective amount of an anti-ErbB2 antibody.

4. The method of claim 3 wherein step (c) further comprises treating the patient with a therapeutically effective amount of a chemotherapeutic agent.

5. The method of claim 1 wherein the tumor overexpresses ErbB2 protein.

6. The method of claim 5 wherein the tumor is selected from the group consisting of a breast tumor, squamous cell tumor, small-cell lung tumor, non-small cell lung tumor, gastrointestinal tumor, pancreatic tumor, glioblastoma, cervical tumor, ovarian tumor, liver tumor, bladder tumor, hepatoma, colon tumor, colorectal tumor, endometrial tumor, salivary gland tumor, kidney tumor, prostate tumor, vulval tumor, thyroid tumor, hepatic tumor, head tumor and neck tumor.

7. The method of claim 6 wherein the tumor is a breast tumor.

8. The method of claim 1 wherein the antibody binds to the extracellular domain of the ErbB2 protein.

9. The method of claim 8 wherein the antibody binds to epitope 4D5 within the ErbB2 extracellular domain sequence.

10. The method of claim 9 wherein the antibody is a humanized 4D5 anti-ErbB2 antibody.

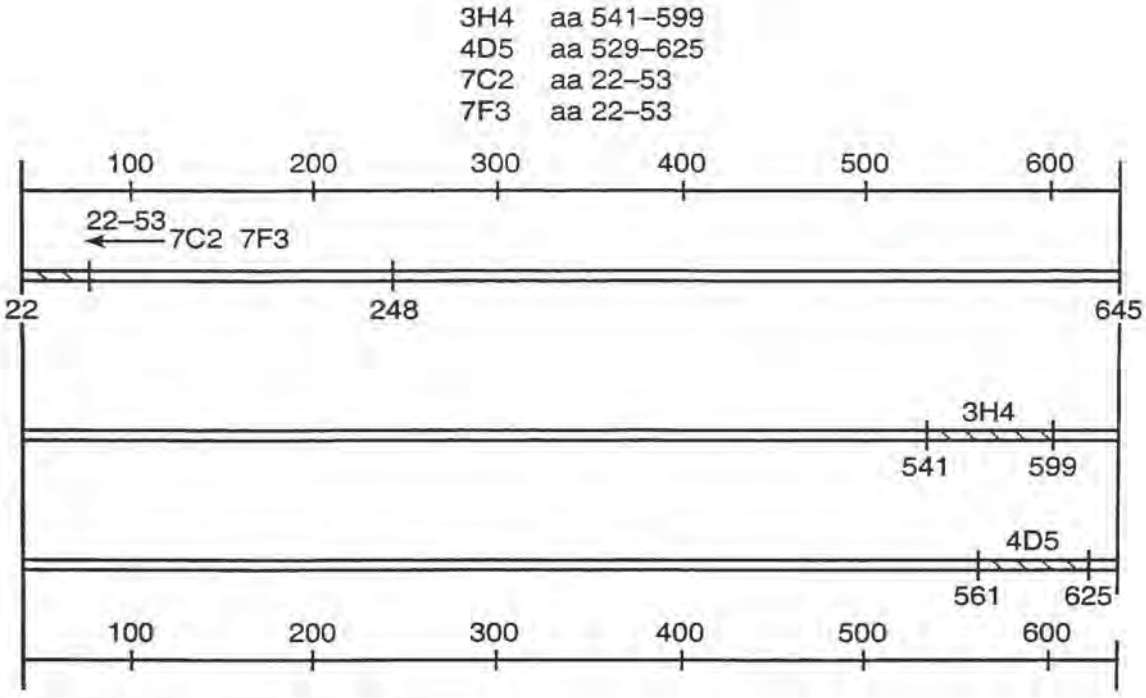
11. The method of claim 2 wherein the chemotherapeutic agent is a taxoid.

12. The method of claim 11 wherein the taxoid is paclitaxel or doxetaxel.

13. The method of claim 4 wherein the chemotherapeutic agent is a taxoid.

14. An article of manufacture comprising a container, a composition within the container comprising an anti-ErbB2 antibody and a package insert instructing the user of the composition to treat a patient essentially according to the method of claim 1.

1 / 1



3H4 epitope (SEQ ID NO:3)

VEECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVAR
541 **599**

4D5 epitope (SEQ ID NO:4)

LPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQP
561 **625**

FIG._1

```
1    MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPA
38   SPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFL
75   QDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDN
112  YALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEI
149  LKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLID
186  TNRSRA
```

FIG._2

Sequence Listing

<110> Genentech, Inc.

<120> TREATMENT WITH ANTI-ErbB2 ANTIBODIES

<130> P1757R1PCT

<141> 2000-05-09

<150> US 60/134,085
<151> 1999-05-14

<160> 4

<210> 1
<211> 166
<212> PRT
<213> Homo sapiens

<400> 1

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Cys Thr Gly Thr Asp Met Lys Leu Arg Leu Pro Ala Ser Pro Glu
 1               5                  10                  15

Thr His Leu Asp Met Leu Arg His Leu Tyr Gln Gly Cys Gln Val
            20                  25                  30

Val Gln Gly Asn Leu Glu Leu Thr Tyr Leu Pro Thr Asn Ala Ser
        35                  40                  45

Leu Ser Phe Leu Gln Asp Ile Gln Glu Val Gln Gly Tyr Val Leu
    50                  55                  60

Ile Ala His Asn Gln Val Arg Gln Val Pro Leu Gln Arg Leu Arg
65                  70                  75

Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr Ala Leu Ala
            80                  85                  90

Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro Val Thr
        95                  100                 105

Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser Leu
    110                 115                 120

Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
125                 130                 135

Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys
140                 145                 150

Asn Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg
155                 160                 165

Ala
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<210> 2
<211> 32
<212> PRT
<213> Homo sapiens

<400> 2

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Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys Leu Arg Leu Pro
```

```
  1               5                  10                  15

Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His Leu Tyr Gln
                20                  25                  30

Gly Cys
```

<210> 3
<211> 59
<212> PRT
<213> Homo sapiens

<400> 3

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Val Glu Glu Cys Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val
  1               5                  10                  15

Asn Ala Arg His Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln
                20                  25                  30

Asn Gly Ser Val Thr Cys Phe Gly Pro Glu Ala Asp Gln Cys Val
                35                  40                  45

Ala Cys Ala His Tyr Lys Asp Pro Pro Phe Cys Val Ala Arg
                50                  55
```

<210> 4
<211> 65
<212> PRT
<213> Homo sapiens

<400> 4

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Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr
  1               5                  10                  15

Cys Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr
                20                  25                  30

Lys Asp Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys
                35                  40                  45

Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu
                50                  55                  60

Gly Ala Cys Gln Pro
                65
```

INTERNATIONAL SEARCH REPORT

Interr. nal Application No
PCT/US 00/12552

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 A61P35/00 //(A61K39/395,31:335)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GOLDENBERG M M: "Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer." CLINICAL THERAPEUTICS, (1999 FEB) 21 (2) 309-18. REF: 28 , XP000918210 page 309 -page 310, paragraph 1 page 314, last paragraph -page 315, right-hand column, paragraph 1 ---	1-14
X	DEES E C ET AL: "Recent advances in systemic therapy for breast cancer." CURRENT OPINION IN ONCOLOGY, (1998 NOV) 10 (6) 517-22. REF: 39 , XP000918214 page 520, right-hand column, paragraph 2 - paragraph 5 ---	1-14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2000	10 OCTOBER 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	von Ballmoos, P

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/12552

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BASELGA J ET AL: "Anti HER2 humanized monoclonal antibody (MAb) alone and in combination with chemotherapy aganst human breast carcinoma xenografts" BREAST CANCER, PROCEEDINGS OF ASCO, VOL. 13 MARCH 1994, ABSTRACT *53, XP000918303 cited in the application the whole document ---	1-14
A	CARTER P ET AL: "HUMANIZATION OF AN ANTI-P185HER2 ANTIBODY FOR HUMAN CANCER THERAPY" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 89, no. 10, 15 May 1992 (1992-05-15), pages 4285-4289, XP000275844 ISSN: 0027-8424 cited in the application the whole document ---	1-14
P,X	WO 99 31140 A (GENENTECH INC) 24 June 1999 (1999-06-24) the whole document -----	1-14

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/12552

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-13 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/US 00/12552

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9931140 A	24-06-1999	AU 1908199 A	05-07-1999

Form PCT/ISA/210 (patent family annex) (July 1992)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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(10) International Publication Number
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(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/811,234 5 June 2006 (05.06.2006) US

(71) Applicants *(for all designated States except US)*: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, CA 94080 (US). **F. HOFFMANN-LA ROCHE, AG** [CH/CH]; Grenzacherstrasse 124, CH-4002 Basel (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **AMLER, Lukas, C.** [DE/US]; 845 Grenada Lane, Foster City, CA 94404 (US). **MOECKS, Joachim** [DE/DE]; Max-Joseph-Strasse 9, 68167 Mannheim (DE). **RABBEE, Nusrat** [US/US]; 2614 Beach Head Way, Richmond, CA 94804 (US). **STRAUSS, Andreas** [DE/DE]; Falkenstrasse 9, 82377 Penzberg (DE).

(74) Agents: **DREGER, Ginger R.** et al.; Heller Ehrman LLP, 275 Middlefield Road, Menlo Park, CA 94025-3506 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: EXTENDING SURVIVAL OF CANCER PATIENTS WITH ELEVATED LEVELS OF EGF OR TGF-ALPHA

(57) Abstract: The present application describes extending survival in a cancer patient, where the patient is producing an elevated level of EGF or TGF-alpha, by treating the patient with a HER dimerization inhibitor, such as pertuzumab.

WO 2007/145862 A2

EXTENDING SURVIVAL OF CANCER PATIENTS WITH ELEVATED LEVELS OF EGF OR TGF-ALPHA

Field of the Invention

The present invention concerns extending survival of a cancer patient, where the patient is producing an elevated level of EGF or TGF-alpha, by treating the patient with a HER dimerization inhibitor, such as pertuzumab.

Background of the Invention

HER Receptors and Antibodies Thereagainst

The HER 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, ErbB1, or HER1), HER2 (ErbB2 or p185^{neu}), HER3 (ErbB3) and HER4 (ErbB4 or tyro2).

EGFR, encoded by the *erb*B1 gene, has been causally implicated in human malignancy. In particular, increased expression of EGFR has been observed in breast, bladder, lung, head, neck and stomach cancer as well as glioblastomas. Increased EGFR receptor expression is often associated with increased production of the EGFR ligand, transforming growth factor alpha (TGF-α), by the same tumor cells resulting in receptor activation by an autocrine stimulatory pathway. Baselga and Mendelsohn *Pharmac. Ther.* 64:127-154 (1994). Monoclonal antibodies directed against the EGFR or its ligands, TGF-α and EGF, have been evaluated as therapeutic agents in the treatment of such malignancies. See, *e.g.*, Baselga and Mendelsohn., *supra*; Masui *et al. Cancer Research* 44:1002-1007 (1984); and Wu *et al. J. Clin. Invest.* 95:1897-1905 (1995).

The second member of the HER family, 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 homolog 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 US 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 HER2 (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); Fukushige *et al.*, *Mol Cell Biol.*, 6:955-958 (1986); Guerin *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.*, 3:254-257 (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). HER2 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)).

Antibodies directed against the rat $p185^{neu}$ and human HER2 protein products have been described.

Drebin and colleagues have raised antibodies against the rat neu gene product, p185neu 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. Patent 5,824,311 issued October 20, 1998.

Hudziak *et al.*, *Mol. Cell. Biol.* 9(3):1165-1172 (1989) describe the generation of a panel of HER2 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 HER2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also U.S. Patent No. 5,677,171 issued October 14, 1997. The HER2 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).

A recombinant humanized version of the murine HER2 antibody 4D5 (huMAb4D5-8, rhuMAb HER2, trastuzumab or HERCEPTIN®; U.S. Patent No. 5,821,337) is clinically active in patients with HER2-overexpressing metastatic breast cancers that have received extensive prior anti-cancer therapy (Baselga *et al.*, *J. Clin. Oncol.* 14:737-744 (1996)). Trastuzumab received

marketing approval from the Food and Drug Administration September 25, 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein.

Other HER2 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. Patent No. 5,783,186; and Klapper *et al. Oncogene* 14:2099-2109 (1997).

Homology screening has resulted in the identification of two other HER receptor family members; HER3 (US Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus *et al. PNAS (USA)* 86:9193-9197 (1989)) and HER4 (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.

The HER receptors are generally found in various combinations in cells and heterodimerization is thought to increase the diversity of cellular responses to a variety of HER ligands (Earp *et al. Breast Cancer Research and Treatment* 35: 115-132 (1995)). EGFR is bound by six different ligands; epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), amphiregulin, heparin binding epidermal growth factor (HB-EGF), betacellulin and epiregulin (Groenen *et al. Growth Factors* 11:235-257 (1994)). A family of heregulin proteins resulting from alternative splicing of a single gene are ligands for HER3 and HER4. The heregulin family includes alpha, beta and gamma heregulins (Holmes *et al., Science*, 256:1205-1210 (1992); U.S. Patent No. 5,641,869; and Schaefer *et al. Oncogene* 15:1385-1394 (1997)); neu differentiation factors (NDFs), glial growth factors (GGFs); acetylcholine receptor inducing activity (ARIA); and sensory and motor neuron derived factor (SMDF). For a review, see Groenen *et al. Growth Factors* 11:235-257 (1994); Lemke, G. *Molec. & Cell. Neurosci.* 7:247-262 (1996) and Lee *et al. Pharm. Rev.* 47:51-85 (1995). Recently three additional HER ligands were identified; neuregulin-2 (NRG-2) which is reported to bind either HER3 or HER4 (Chang *et al. Nature* 387 509-512 (1997); and Carraway *et al Nature* 387:512-516 (1997)); neuregulin-3 which binds HER4 (Zhang *et al. PNAS* (USA) 94(18):9562-7 (1997)); and neuregulin-4 which binds HER4 (Harari *et al. Oncogene* 18:2681-89 (1999)) HB-EGF, betacellulin and epiregulin also bind to HER4.

While EGF and TGFα do not bind HER2, EGF stimulates EGFR and HER2 to form a heterodimer, which activates EGFR and results in transphosphorylation of HER2 in the heterodimer. Dimerization and/or transphosphorylation appears to activate the HER2 tyrosine

kinase. See Earp *et al.*, *supra*. Likewise, when HER3 is co-expressed with HER2, an active signaling complex is formed and antibodies directed against HER2 are capable of disrupting this complex (Sliwkowski *et al.*, *J. Biol. Chem.*, 269(20):14661-14665 (1994)). Additionally, the affinity of HER3 for heregulin (HRG) is increased to a higher affinity state when co-expressed with HER2. See also, Levi *et al.*, *Journal of Neuroscience* 15: 1329-1340 (1995); Morrissey *et al.*, *Proc. Natl. Acad. Sci. USA* 92: 1431-1435 (1995); and Lewis *et al.*, *Cancer Res.*, 56:1457-1465 (1996) with respect to the HER2-HER3 protein complex. HER4, like HER3, forms an active signaling complex with HER2 (Carraway and Cantley, *Cell* 78:5-8 (1994)).

Patent publications related to HER antibodies include: US 5,677,171, US 5,720,937, US 5,720,954, US 5,725,856, US 5,770,195, US 5,772,997, US 6,165,464, US 6,387,371, US 6,399,063, US2002/0192211A1, US 6,015,567, US 6,333,169, US 4,968,603, US 5,821,337, US 6,054,297, US 6,407,213, US 6,719,971, US 6,800,738, US2004/0236078A1, US 5,648,237, US 6,267,958, US 6,685,940, US 6,821,515, WO98/17797, US 6,127,526, US 6,333,398, US 6,797,814, US 6,339,142, US 6,417,335, US 6,489,447, WO99/31140, US2003/0147884A1, US2003/0170234A1, US2004/0037823A1, US2005/0002928A1, US 6,573,043, US 6,905,830, US2003/0152987A1, WO99/48527, US2002/0141993A1, US2005/0244417A1, US Patent No. 6,949,245, US2003/0086924, US2004/0013667A1, WO00/69460, US2003/0170235A1, US 7,041,292, WO01/00238, US2006/0083739, WO01/15730, US 6,627,196B1, US6,632,979B1, WO01/00244, US2002/0001587A1, US2002/0090662A1, US6,984,494B2, WO01/89566, US2002/0064785, US2003/0134344, WO 2005/099756, US2006/0013819, WO2006/07398A1, US2006/0018899, WO 2006/33700, US2006/0088523, US 2006/0034840, WO 04/24866, US2004/0082047, US2003/0175845A1, WO03/087131, US2003/0228663, WO2004/008099A2, US2004/0106161, WO2004/048525, US2004/0258685A1, WO 2005/16968, US2005/0038231A1US 5,985,553, US 5,747,261, US 4,935,341, US 5,401,638, US 5,604,107, WO 87/07646, WO 89/10412, WO 91/05264, EP 412,116 B1, EP 494,135 B1, US 5,824,311, EP 444,181 B1, EP 1,006,194 A2, US 2002/0155527A1, WO 91/02062, US 5,571,894, US 5,939,531, EP 502,812 B1, WO 93/03741, EP 554,441 B1, EP 656,367 A1, US 5,288,477, US 5,514,554, US 5,587,458, WO 93/12220, WO 93/16185, US 5,877,305, WO 93/21319, WO 93/21232, US 5,856,089, WO 94/22478, US 5,910,486, US 6,028,059, WO 96/07321, US 5,804,396, US 5,846,749, EP 711,565, WO 96/16673, US 5,783,404, US 5,977,322, US 6,512,097, WO 97/00271, US 6,270,765, US 6,395,272, US 5,837,243, WO 96/40789, US 5,783,186, US 6,458,356, WO 97/20858, WO 97/38731, US 6,214,388, US 5,925,519, WO 98/02463, US 5,922,845, WO 98/18489, WO 98/33914, US 5,994,071, WO 98/45479, US 6,358,682 B1, US 2003/0059790, WO 99/55367, WO 01/20033, US 2002/0076695 A1, WO 00/78347, WO 01/09187, WO 01/21192, WO 01/32155, WO 01/53354, WO 01/56604, WO 01/76630, WO02/05791, WO 02/11677, US 6,582,919, US2002/0192652A1, US

2003/0211530A1, WO 02/44413, US 2002/0142328, US 6,602,670 B2, WO 02/45653, WO 02/055106, US 2003/0152572, US 2003/0165840, WO 02/087619, WO 03/006509, WO03/012072, WO 03/028638, US 2003/0068318, WO 03/041736, EP 1,357,132, US 2003/0202973, US 2004/0138160, US 5,705,157, US 6,123,939, EP 616,812 B1, US 2003/0103973, US 2003/0108545, US 6,403,630 B1, WO 00/61145, WO 00/61185, US 6,333,348 B1, WO 01/05425, WO 01/64246, US 2003/0022918, US 2002/0051785 A1, US 6,767,541, WO 01/76586, US 2003/0144252, WO 01/87336, US 2002/0031515 A1, WO 01/87334, WO 02/05791, WO 02/09754, US 2003/0157097, US 2002/0076408, WO 02/055106, WO 02/070008, WO 02/089842 and WO 03/86467.

Diagnostics

Patients treated with the HER2 antibody trastuzumab are selected for therapy based on HER2 overexpression/amplification. See, for example, WO99/31140 (Paton *et al.*), US2003/0170234A1 (Hellmann, S.), and US2003/0147884 (Paton *et al.*); as well as WO01/89566, US2002/0064785, and US2003/0134344 (Mass *et al.*). See, also, US Patent No. 6,573,043, US Patent No. 6,905,830, and US2003/0152987, Cohen *et al.*, concerning immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) for detecting HER2 overexpression and amplification.

WO2004/053497 and US2004/024815A1 (Bacus *et al.*), as well as US 2003/0190689 (Crosby and Smith), refer to determining or predicting response to trastuzumab therapy. US2004/013297A1 (Bacus *et al.*) concerns determining or predicting response to ABX0303 EGFR antibody therapy. WO2004/000094 (Bacus *et al.*) is directed to determining response to GW572016, a small molecule, EGFR-HER2 tyrosine kinase inhibitor. WO2004/063709, Amler *et al.*, refers to biomarkers and methods for determining sensitivity to EGFR inhibitor, erlotinib HCl. US2004/0209290 and WO04/065583, Cobleigh *et al.*, concern gene expression markers for breast cancer prognosis. See, also, WO03/078662 (Baker *et al.*), and WO03/040404 (Bevilacqua et al.). WO02/44413 (Danenberg, K.) refers to determining EGFR and HER2 gene expression for determining a chemotherapeutic regimen.

Patients treated with pertuzumab can be selected for therapy based on HER activation or dimerization. Patent publications concerning pertuzumab and selection of patients for therapy therewith include: US Patent No. 6,949,245, WO01/00245, US2005/0208043, US2005/0238640, US2006/0034842, and US2006/0073143 (Adams *et al.*); US2003/0086924 (Sliwkowski, M.); US2004/0013667A1 (Sliwkowski, M.); as well as WO2004/008099A2, and US2004/0106161 (Bossenmaier *et al.*).

Cronin *et al. Am. J. Path.* 164(1): 35-42 (2004) describes measurement of gene expression in archival paraffin-embedded tissues. Ma *et al. Cancer Cell* 5:607-616 (2004)

describes gene profiling by gene oligonucleotide microarray using isolated RNA from tumor-tissue sections taken from archived primary biopsies.

Pertuzumab (also known as recombinant human monoclonal antibody 2C4; OMNITARG™, Genentech, Inc, South San Francisco) represents the first in a new class of agents known as HER dimerization inhibitors (HDI) and functions to inhibit the ability of HER2 to form active heterodimers with other HER receptors (such as EGFR/HER1, HER3 and HER4) and is active irrespective of HER2 expression levels. See, for example, Harari and Yarden *Oncogene* 19:6102-14 (2000); Yarden and Sliwkowski. *Nat Rev Mol Cell Biol* 2:127-37 (2001); Sliwkowski *Nat Struct Biol* 10:158-9 (2003); Cho *et al. Nature* 421:756-60 (2003); and Malik *et al. Pro Am Soc Cancer Res* 44:176-7 (2003).

Pertuzumab blockade of the formation of HER2-HER3 heterodimers in tumor cells has been demonstrated to inhibit critical cell signaling, which results in reduced tumor proliferation and survival (Agus *et al. Cancer Cell* 2:127-37 (2002)).

Pertuzumab has undergone testing as a single agent in the clinic with a phase Ia trial in patients with advanced cancers and phase II trials in patients with ovarian cancer and breast cancer as well as lung and prostate cancer. In a Phase I study, patients with incurable, locally advanced, recurrent or metastatic solid tumors that had progressed during or after standard therapy were treated with pertuzumab given intravenously every 3 weeks. Pertuzumab was generally well tolerated. Tumor regression was achieved in 3 of 20 patients evaluable for response. Two patients had confirmed partial responses. Stable disease lasting for more than 2.5 months was observed in 6 of 21 patients (Agus *et al. Pro Am Soc Clin Oncol* 22:192 (2003)). At doses of 2.0-15 mg/kg, the pharmacokinetics of pertuzumab was linear, and mean clearance ranged from 2.69 to 3.74 mL/day/kg and the mean terminal elimination half-life ranged from 15.3 to 27.6 days. Antibodies to pertuzumab were not detected (Allison *et al. Pro Am Soc Clin Oncol* 22:197 (2003)).

Summary of the Invention

The present invention provides the clinical data from human cancer patients treated with a HER dimerization inhibitor, pertuzumab. Patients were evaluated for expression levels of various serum biomarkers and the correlation between such expression levels and clinical benefit in response to treatment with trastuzumab was assessed. The clinical data indicated that patients with ovarian cancer who produce elevated levels of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha) showed survival benefits relative to patients with normal EGF or TGF-alpha levels, in response to pertuzumab treatment. Similar benefits are expected in another ongoing clinical trial, including patients with platinum-resistant ovarian cancer, primary peritoneal and fallopian tube cancer.

Accordingly, in one aspect the invention concerns a method for extending survival of a cancer patient comprising administering a HER dimerization inhibitor to the patient in an amount which extends survival of the patient, wherein the patient is determined to produce an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha), and the cancer is selected from the group consisting of ovarian cancer, peritoneal cancer and fallopian rube cancer.

In another aspect, the invention concerns a method for extending survival of a patient with ovarian, peritoneal, or fallopian tube cancer comprising administering pertuzumab to the patient in an amount which extends survival of the patent, wherein the patient is determined to produce an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha).

In a further aspect, the invention concerns a method for extending progression free survival (PFS) of a patient with ovarian, peritoneal, or fallopian tube cancer comprising administering pertuzumab to the patient in an amount which extends PFS in the patent, wherein the patient's serum is determined to have an elevated level of epidermal growth factor (EGF) therein.

In a still further aspect, the invention concerns a method for extending progression free survival (PFS) of a patient with ovarian, peritoneal, or fallopian tube cancer comprising administering pertuzumab to the patient in an amount which extends PFS in the patent, wherein the patient's serum is determined to have an elevated level of epidermal growth factor (EGF) and transforming growth factor alpha (TGF-alpha) therein.

In an additional aspect, the invention concerns a method of selecting a patient for treatment with a HER dimerization inhibitor, comprising treating the patient with the HER dimerization inhibitor if the patient is determined to produce an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha).

For all aspects, in a particular embodiment, the patient is found to have an elevated level of EGF in the serum of the patient.

In another embodiment, the patient is found to have an elevated level of TGF-alpha in serum of the patient.

In another embodiment, the HER dimerization inhibitor is a HER2 dimerization inhibitor.

In yet another embodiment, the HER dimerization inhibitor inhibits HER heterodimerization.

In a further embodiment, the HER dimerization inhibitor is a HER antibody, which may, for example, bind to a HER receptor selected from the group consisting of EGFR, HER2, and HER3.

In a particular embodiment, the antibody binds to HER2, such as to Domain II of HER2 extracellular domain, or to a junction between domains I, II and III of HER2 extracellular domain.

In a specific embodiment, the HER dimerization inhibitor is pertuzumab.

The cancer can, for example, be advanced, refractory or recurrent ovarian cancer, platinum resistant ovarian cancer, primary peritoneal or fallopian tube cancer

The HER dimerization inhibitor may be administered as a single anti-tumor agent, or in combination with a second therapeutic agent to the patient.

The second therapeutic agent may, for example, be a chemotherapeutic agent, a HER antibody, antibody directed against a tumor associated antigen, an anti-hormonal compound, a cardioprotectant, a cytokine, an EGFR-targeted drug, an anti-angiogenic agent, a tyrosine kinase inhibitor, a COX inhibitor, a non-steroidal anti-inflammatory drug, a farnesyl transferase inhibitor, an antibody that binds oncofetal protein CA 125, HER2 vaccine, a HER targeting therapy, a Raf or ras inhibitor, a liposomal doxorubicin, a topotecan, a taxane, a dual tyrosine kinase inhibitor, TLK286, EMD-7200, a medicament that treats nausea, a medicament that prevents or treats skin rash or standard acne therapy, a medicament that treats or prevents diarrhea, a body temperature-reducing medicament, or a hematopoietic growth factor.

In a particular embodiment, the second therapeutic agent is a chemotherapeutic agent, such as an antimetabolite chemotherapeutic agent, e.g. gemcitabine, trastuzumab, erlotinib, or bevacizumab.

The clinical benefit is preferably measured in terms of survival, including overall survival (OS) and progression free survival (PFS), preferably PFS.

In another aspect, the invention concerns a kit comprising a HER dimerization inhibitor and a package insert or label indicating a clinical benefit for the HER dimerization inhibitor if the patient to be treated produces an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha), wherein the clinical benefit preferably is extended survival, in particular extended PFS.

In a further aspect, the invention concerns a method of promoting a HER dimerization inhibitor to treat patients producing an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha), where the promotion can take any forms, including the form of a written material, such a package insert.

Brief Description of the Drawings

Figure 1 provides a schematic of the HER2 protein structure, and amino acid sequences for Domains I-IV (SEQ ID Nos.19-22, respectively) of the extracellular domain thereof.

Figures 2A and 2B depict alignments of the amino acid sequences of the variable light (V_L) (Fig. 2A) and variable heavy (V_H) (Fig. 2B) domains of murine monoclonal antibody 2C4

(SEQ ID Nos. 1 and 2, respectively); V_L and V_H domains of variant 574/pertuzumab (SEQ ID Nos. 3 and 4, respectively), and human V_L and V_H consensus frameworks (hum κ1, light kappa subgroup I; humIII, heavy subgroup III) (SEQ ID Nos. 5 and 6, respectively). Asterisks identify differences between variable domains of pertuzumab and murine monoclonal antibody 2C4 or between variable domains of pertuzumab and the human framework. Complementarity Determining Regions (CDRs) are in brackets.

Figures 3A and 3B show the amino acid sequences of pertuzumab light chain (Fig. 3A; SEQ ID NO. 13) and heavy chain (Fig. 3B; SEQ ID No. 14). CDRs are shown in bold. Calculated molecular mass of the light chain and heavy chain are 23,526.22 Da and 49,216.56 Da (cysteines in reduced form). The carbohydrate moiety is attached to Asn 299 of the heavy chain.

Figure 4 depicts, schematically, binding of 2C4 at the heterodimeric binding site of HER2, thereby preventing heterodimerization with activated EGFR or HER3.

Figure 5 depicts coupling of HER2/HER3 to the MAPK and Akt pathways.

Figure 6 compares various activities of trastuzumab and pertuzumab.

Figures 7A and 7B show the amino acid sequences of trastuzumab light chain (Fig. 7A; SEQ ID No. 15) and heavy chain (Fig. 7B; SEQ ID No. 16), respectively.

Figures 8A and 8B depict a variant pertuzumab light chain sequence (Fig. 8A; SEQ ID No. 17) and a variant pertuzumab heavy chain sequence (Fig. 8B; SEQ ID No. 18), respectively.

Figure 9 depicts Spearman correlation between biomarkers HER2, TGF-alpha, amphiregulin, and EGF.

Figure 10 represents mean/correlation of markers with clinical covariates.

Figure 11 shows cutoff determination using progression free survival (PFS) for HER2, TGF-alpha, amphiregulin, and EGF.

Figure 12 shows cutoff determination using overall survival (OS) for HER2, TGF-alpha, amphiregulin, and EGF.

Figure 13 reflects distribution of patients according to cutoffs.

Figure 14 depicts KapLan Meir PFS and OS curves separated by 3 marker cutoff determined in the univariate analysis for the HER2 marker.

Figure 15 depicts KapLan Meir PFS and OS curves separated by 3 marker cutoff determined in the univariate analysis for the TGF-alpha marker.

Figure 16 depicts KapLan Meir PFS and OS curves separated by 3 marker cutoff determined in the univariate analysis for the EGF marker.

Detailed Description of the Preferred Embodiments

I. Definitions

"Survival" refers to the patient remaining alive, and includes overall survival as well as progression free survival.

"Overall survival" refers to the patient remaining alive for a defined period of time, such as 1 year, 5 years, etc from the time of diagnosis or treatment.

"Progression free survival" refers to the patient remaining alive, without the cancer progressing or getting worse.

By "extending survival" is meant increasing overall or progression free survival in a treated patient relative to an untreated patient (*i.e.* relative to a patient not treated with a HER dimerization inhibitor, such as pertuzumab), or relative to a patient who does not display HER activation, and/or relative to a patient treated with an approved anti-tumor agent (such as topotecan or liposomal doxorubicin, where the cancer is ovarian cancer).

Herein "time to disease progression" or "TTP" refer to the time, generally measured in weeks or months, from the time of initial treatment (*e.g.* with a HER dimerization inhibitor, such as pertuzumab), until the cancer progresses or worsens. Such progression can be evaluated by the skilled clinician. In the case of ovarian cancer, for instance, progression can be evaluated by RECIST (see, for example, Therasse *et al., J. Nat. Cancer Inst.* 92(3): 205-216 (2000)).

By "extending TTP" is meant increasing the time to disease progression in a treated patient relative to an untreated patient (*i.e.* relative to a patient not treated with a HER dimerization inhibitor, such as pertuzumab), or relative to a patient who does not display HER activation, and/or relative to a patient treated with an approved anti-tumor agent (such as topotecan or liposomal doxorubicin, where the cancer is ovarian cancer).

An "objective response" refers to a measurable response, including complete response (CR) or partial response (PR).

By "complete response" or "CR" is intended the disappearance of all signs of cancer in response to treatment. This does not always mean the cancer has been cured.

"Partial response" or "PR" refers to a decrease in the size of one or more tumors or lesions, or in the extent of cancer in the body, in response to treatment.

A "HER receptor" is a receptor protein tyrosine kinase which belongs to the HER receptor family and includes EGFR, HER2, HER3 and HER4 receptors. The HER receptor will generally comprise an extracellular domain, which may bind an HER ligand and/or dimerize with another HER receptor molecule; 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 HER receptor may be a "native sequence" HER receptor or an "amino acid sequence variant" thereof. Preferably the HER receptor is native sequence human HER receptor.

The terms "ErbB1," "HER1", "epidermal growth factor receptor" and "EGFR" are used interchangeably herein and refer to 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)). *erb*B1 refers to the gene encoding the EGFR protein product.

The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to 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). The term "*erb*B2" refers to the gene encoding human ErbB2 and "*neu*@ refers to the gene encoding rat $p185^{neu}$. Preferred HER2 is native sequence human HER2.

Herein, "HER2 extracellular domain" or "HER2 ECD" refers to a domain of HER2 that is outside of a cell, either anchored to a cell membrane, or in circulation, including fragments thereof. In one embodiment, the extracellular domain of HER2 may comprise four domains: "Domain I" (amino acid residues from about 1-195; SEQ ID NO:19), "Domain II" (amino acid residues from about 196-319; SEQ ID NO:20), "Domain III" (amino acid residues from about 320-488: SEQ ID NO:21), and "Domain IV" (amino acid residues from about 489-630; SEQ ID NO:22) (residue numbering without signal peptide). See Garrett *et al. Mol. Cell.*. 11: 495-505 (2003), Cho *et al. Nature* 421: 756-760 (2003), Franklin *et al. Cancer Cell* 5:317-328 (2004), and Plowman *et al. Proc. Natl. Acad. Sci.* 90:1746-1750 (1993), as well as Fig. 1 herein.

"ErbB3" and "HER3" refer to the receptor polypeptide as disclosed, for example, in US Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus *et al. PNAS (USA)* 86:9193-9197 (1989).

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), including isoforms thereof, *e.g.*, as disclosed in WO99/19488, published April 22, 1999.

By "HER ligand" is meant a polypeptide which binds to and/or activates a HER receptor. The HER ligand of particular interest herein is a native sequence human HER ligand such as epidermal growth factor (EGF) (Savage *et al., J. Biol. Chem.* 247:7612-7621 (1972)); transforming growth factor alpha (TGF-α) (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)); neuregulin-4 (NRG-4) (Harari *et al. Oncogene* 18:2681-89 (1999)); and cripto (CR-1) (Kannan *et al. J. Biol. Chem.* 272(6):3330-3335 (1997)). HER ligands which bind EGFR include EGF, TGF-α, amphiregulin, betacellulin, HB-EGF and epiregulin. HER ligands which bind HER3 include heregulins. HER ligands capable of binding HER4 include betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3, NRG-4, and heregulins.

"Heregulin" (HRG) when used herein refers to a polypeptide encoded by the heregulin gene product as disclosed in U.S. Patent No. 5,641,869, or Marchionni *et al.*, *Nature*, 362:312-318 (1993). Examples of heregulins include heregulin-α, heregulin-β1, heregulin-β2 and heregulin-β3 (Holmes *et al.*, *Science*, 256:1205-1210 (1992); and U.S. Patent No. 5,641,869); *neu* differentiation factor (NDF) (Peles *et al. Cell* 69: 205-216 (1992)); acetylcholine receptor-inducing activity (ARIA) (Falls *et al. Cell* 72:801-815 (1993)); glial growth factors (GGFs) (Marchionni *et al.*, *Nature*, 362:312-318 (1993)); sensory and motor neuron derived factor (SMDF) (Ho *et al. J. Biol. Chem.* 270:14523-14532 (1995)); γ-heregulin (Schaefer *et al. Oncogene* 15:1385-1394 (1997)).

A "HER dimer" herein is a noncovalently associated dimer comprising at least two HER receptors. Such complexes may form when a cell expressing two or more HER receptors is exposed to an HER 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. Other proteins, such as a cytokine receptor subunit (*e.g.* gp130) may be associated with the dimer. Preferably, the HER dimer comprises HER2.

A "HER heterodimer" herein is a noncovalently associated heterodimer comprising at least two different HER receptors, such as EGFR-HER2, HER2-HER3 or HER2-HER4 heterodimers.

A "HER inhibitor" is an agent which interferes with HER activation or function. Examples of HER inhibitors include HER antibodies (*e.g.* EGFR, HER2, HER3, or HER4 antibodies); EGFR-targeted drugs; small molecule HER antagonists; HER tyrosine kinase inhibitors; HER2 and EGFR dual tyrosine kinase inhibitors such as lapatinib/GW572016; antisense molecules (see, for example, WO2004/87207); and/or agents that bind to, or interfere with function of, downstream signaling molecules, such as MAPK or Akt (see Fig. 5). Preferably, the HER inhibitor is an antibody or small molecule which binds to a HER receptor.

A "HER dimerization inhibitor" is an agent which inhibits formation of a HER dimer or HER heterodimer. Preferably, the HER dimerization inhibitor is an antibody, for example an antibody which binds to HER2 at the heterodimeric binding site thereof. The most preferred HER dimerization inhibitor herein is pertuzumab or MAb 2C4. Binding of 2C4 to the heterodimeric binding site of HER2 is illustrated in Fig. 4. Other examples of HER dimerization inhibitors

include antibodies which bind to EGFR and inhibit dimerization thereof with one or more other HER receptors (for example EGFR monoclonal antibody 806, MAb 806, which binds to activated or "untethered" EGFR; see Johns *et al., J. Biol. Chem.* 279(29):30375-30384 (2004)); antibodies which bind to HER3 and inhibit dimerization thereof with one or more other HER receptors; antibodies which bind to HER4 and inhibit dimerization thereof with one or more other HER receptors; peptide dimerization inhibitors (US Patent No. 6,417,168); antisense dimerization inhibitors; etc.

A "HER2 dimerization inhibitor" is an agent that inhibits formation of a dimer or heterodimer comprising HER2.

A "HER antibody" is an antibody that binds to a HER receptor. Optionally, the HER antibody further interferes with HER activation or function. Preferably, the HER antibody binds to the HER2 receptor. A HER2 antibody of particular interest herein is pertuzumab. Another example of a HER2 antibody is trastuzumab. Examples of EGFR antibodies include cetuximab and ABX0303.

"HER activation" refers to activation, or phosphorylation, of any one or more HER receptors. Generally, HER activation results in signal transduction (*e.g.* that caused by an intracellular kinase domain of a HER receptor phosphorylating tyrosine residues in the HER receptor or a substrate polypeptide). HER activation may be mediated by HER ligand binding to a HER dimer comprising the HER receptor of interest. HER ligand binding to a HER dimer may activate a kinase domain of one or more of the HER receptors in the dimer and thereby results in phosphorylation of tyrosine residues in one or more of the HER receptors and/or phosphorylation of tyrosine residues in additional substrate polypeptides(s), such as Akt or MAPK intracellular kinases, see, Fig. 5, for example.

"Phosphorylation" refers to the addition of one or more phosphate group(s) to a protein, such as a HER receptor, or substrate thereof.

An antibody which "inhibits HER dimerization" is an antibody which inhibits, or interferes with, formation of a HER dimer. Preferably, such an antibody binds to HER2 at the heterodimeric binding site thereof. The most preferred dimerization inhibiting antibody herein is pertuzumab or MAb 2C4. Binding of 2C4 to the heterodimeric binding site of HER2 is illustrated in Fig. 4. Other examples of antibodies which inhibit HER dimerization include antibodies which bind to EGFR and inhibit dimerization thereof with one or more other HER receptors (for example EGFR monoclonal antibody 806, MAb 806, which binds to activated or Auntethered@ EGFR; see Johns *et al., J. Biol. Chem.* 279(29):30375-30384 (2004)); antibodies which bind to HER3 and inhibit dimerization thereof with one or more other HER receptors; and antibodies which bind to HER4 and inhibit dimerization thereof with one or more other HER receptors.

An antibody which "blocks ligand activation of a HER receptor more effectively than trastuzumab" is one which reduces or eliminates HER ligand activation of HER receptor(s) or HER dimer(s) more effectively (for example at least about 2-fold more effectively) than trastuzumab. Preferably, such an antibody blocks HER ligand activation of a HER receptor at least about as effectively as murine monoclonal antibody 2C4 or a Fab fragment thereof, or as pertuzumab or a Fab fragment thereof. One can evaluate the ability of an antibody to block ligand activation of a HER receptor by studying HER dimers directly, or by evaluating HER activation, or downstream signaling, which results from HER dimerization, and/or by evaluating the antibody-HER2 binding site, etc. Assays for screening for antibodies with the ability to inhibit ligand activation of a HER receptor more effectively than trastuzumab are described in Agus *et al. Cancer Cell* 2: 127-137 (2002) and US Patent No. 6,949,245 (Adams *et al.*). By way of example only, one may assay for: inhibition of HER dimer formation (see, *e.g.*, Fig. 1A-B of Agus *et al. Cancer Cell* 2: 127-137 (2002); and US Patent No. 6,949,245); reduction in HER ligand activation of cells which express HER dimers (US Patent No. 6,949,245 and Fig. 2A-B of Agus *et al. Cancer Cell* 2: 127-137 (2002), for example); blocking of HER ligand binding to cells which express HER dimers (US Patent No. 6,949,245, and Fig. 2E of Agus *et al. Cancer Cell* 2: 127-137 (2002), for example); cell growth inhibition of cancer cells (*e.g.* MCF7, MDA-MD-134, ZR-75-1, MD-MB-175, T-47D cells) which express HER dimers in the presence (or absence) of HER ligand (US Patent No. 6,949,245and Figs. 3A-D of Agus *et al. Cancer Cell* 2: 127-137 (2002), for instance); inhibition of downstream signaling (for instance, inhibition of HRG-dependent AKT phosphorylation or inhibition of HRG- or TGFα- dependent MAPK phosphorylation) (see, US Patent No. 6,949,245, and Fig. 2C-D of Agus et al. *Cancer Cell* 2: 127-137 (2002), for example). One may also assess whether the antibody inhibits HER dimerization by studying the antibody-HER2 binding site, for instance, by evaluating a structure or model, such as a crystal structure, of the antibody bound to HER2 (See, for example, Franklin *et al. Cancer Cell* 5:317-328 (2004)).

A "heterodimeric binding site" on HER2, refers to a region in the extracellular domain of HER2 that contacts, or interfaces with, a region in the extracellular domain of EGFR, HER3 or HER4 upon formation of a dimer therewith. The region is found in Domain II of HER2. Franklin *et al. Cancer Cell* 5:317-328 (2004).

The HER2 antibody may "inhibit HRG-dependent AKT phosphorylation" and/or inhibit "HRG- or TGFα-dependent MAPK phosphorylation" more effectively (for instance at least 2-fold more effectively) than trastuzumab (see Agus *et al. Cancer Cell* 2: 127-137 (2002) and US Patent No. 6,949,245, by way of example).

The HER2 antibody may be one which, like pertuzumab, does "not inhibit HER2 ectodomain cleavage" (Molina *et al. Cancer Res.* 61:4744-4749(2001)). Trastuzumab, on the other hand, can inhibit HER2 ectodomain cleavage.

A HER2 antibody that "binds to a heterodimeric binding site" of HER2, binds to residues in domain II (and optionally also binds to residues in other of the domains of the HER2 extracellular domain, such as domains I and III), and can sterically hinder, at least to some extent, formation of a HER2-EGFR, HER2-HER3, or HER2-HER4 heterodimer. Franklin *et al. Cancer Cell* 5:317-328 (2004) characterize the HER2-pertuzumab crystal structure, deposited with the RCSB Protein Data Bank (ID Code IS78), illustrating an exemplary antibody that binds to the heterodimeric binding site of HER2.

An antibody that "binds to domain II" of HER2 binds to residues in domain II and optionally residues in other domain(s) of HER2, such as domains I and III. Preferably the antibody that binds to domain II binds to the junction between domains I, II and III of HER2.

Protein "expression" refers to conversion of the information encoded in a gene into messenger RNA (mRNA) and then to the protein.

Herein, a sample or cell that "expresses" a protein of interest (such as a HER receptor or HER ligand) is one in which mRNA encoding the protein, or the protein, including fragments thereof, is determined to be present in the sample or cell.

The technique of "polymerase chain reaction" or "PCR" as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 51: 263 (1987); Erlich, ed., PCR Technology, (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid (DNA or RNA) as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

"Quantitative real time polymerase chain reaction" or "qRT-PCR" refers to a form of PCR wherein the amount of PCR product is measured at each step in a PCR reaction. This

technique has been described in various publications including Cronin *et al.*, *Am. J. Pathol.* 164(1):35-42 (2004); and Ma *et al.*, *Cancer Cell* 5:607-616 (2004).

The term "microarray" refers to an ordered arrangement of hybridizable array elements, preferably polynucleotide probes, on a substrate.

The term "polynucleotide," when used in singular or plural, generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as defined herein include, without limitation, single- and double-stranded DNA, DNA including single- and double-stranded regions, single- and double-stranded RNA, and RNA including single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or include single- and double-stranded regions. In addition, the term "polynucleotide" as used herein refers to triple- stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. The term "polynucleotide" specifically includes cDNAs. The term includes DNAs (including cDNAs) and RNAs that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritiated bases, are included within the term "polynucleotides" as defined herein. In general, the term "polynucleotide" embraces all chemically, enzymatically and/or metabolically modified forms of unmodified polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells.

The term "oligonucleotide" refers to a relatively short polynucleotide, including, without limitation, single-stranded deoxyribonucleotides, single- or double-stranded ribonucleotides, RNA:DNA hybrids and double- stranded DNAs. Oligonucleotides, such as single- stranded DNA probe oligonucleotides, are often synthesized by chemical methods, for example using automated oligonucleotide synthesizers that are commercially available. However, oligonucleotides can be made by a variety of other methods, including in vitro recombinant DNA-mediated techniques and by expression of DNAs in cells and organisms.

The phrase "gene amplification" refers to a process by which multiple copies of a gene or gene fragment are formed in a particular cell or cell line. The duplicated region (a stretch of amplified DNA) is often referred to as "amplicon." Usually, the amount of the messenger RNA (mRNA) produced also increases in the proportion of the number of copies made of the particular gene expressed.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, typically: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C.; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C.; or (3) employ 50% formamide, 5×SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5× Denhardt's solution, sonicated salmon sperm DNA (50 &gr;g/ml), 0.1% SDS, and 10% dextran sulfate at 42°C., with washes at 42°C. in 0.2×SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C., followed by a high- stringency wash consisting of 0.1×SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (*e.g.*, temperature, ionic strength and % SDS) less stringent that those described above. An example of moderately stringent conditions is overnight incubation at 37°C. in a solution comprising: 20% formamide, 5×SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5× Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1×SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (*e.g.*, HER receptor or HER ligand) derived from nature, including naturally occurring or allelic variants. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can

have the amino acid sequence of naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

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

The term "monoclonal antibody" as used herein refers to an antibody from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope(s), except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Such monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones or recombinant DNA clones. It should be understood that the selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, *etc.*, and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, the monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins. 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 a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler *et al.*, *Nature*, 256:495 (1975); Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas 563-681, (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567), phage display technologies (see, *e.g.*, Clackson *et al.*, *Nature*, 352:624-628 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991); Sidhu *et al.*, *J. Mol. Biol.* 338(2):299-310 (2004); Lee *et al.*, *J.Mol.Biol.*340(5):1073-1093 (2004); Fellouse, *Proc. Nat. Acad. Sci. USA* 101(34):12467-12472 (2004); and Lee *et al. J. Immunol. Methods* 284(1-2):119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human

immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al., Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al., Nature*, 362:255-258 (1993); *Bruggemann et al., Year in Immuno.*, 7:33 (1993); U.S. Patent Nos. 5,545,806; 5,569,825; 5,591,669 (all of GenPharm); U.S. Patent No. 5,545,807; WO 1997/17852; U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al., Bio/Technology*, 10: 779-783 (1992); Lonberg *et al., Nature*, 368: 856-859 (1994); Morrison, *Nature*, 368: 812-813 (1994); Fishwild *et al., Nature Biotechnology*, 14: 845-851 (1996); Neuberger, *Nature Biotechnology*, 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.*, 13: 65-93 (1995)).

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. Patent No. 4,816,567; and Morrison *et al., Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include Aprimatized® 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, as well as "humanized" antibodies.

"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 HER2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 or trastuzumab (HERCEPTIN®) as described in Table 3 of U.S. Patent 5,821,337 expressly incorporated herein by reference; humanized 520C9 (WO93/21319); and humanized 2C4 antibodies such as pertuzumab as described herein.

For the purposes herein, "trastuzumab," "HERCEPTIN®," and "huMAb4D5-8" refer to an antibody comprising the light and heavy chain amino acid sequences in SEQ ID NOS. 15 and 16, respectively.

Herein, "pertuzumab" and "OMNITARG™" refer to an antibody comprising the light and heavy chain amino acid sequences in SEQ ID NOS. 13 and 14, respectively.

Differences between trastuzumab and pertuzumab functions are illustrated in Fig. 6.

An "intact antibody" herein is one which comprises two antigen binding regions, and an Fc region. Preferably, the intact antibody has a functional Fc region.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding 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).

"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" refers to the fact that certain portions of the 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.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab= fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue.

Unless indicated otherwise, herein the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991), expressly incorporated herein by reference. The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

A "functional Fc region" possesses an "effector function" of a native sequence Fc region. Exemplary "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. Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.* an antibody variable domain) and can be assessed using various assays as herein disclosed, for example.

A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, *e.g.* from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent

polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different Aclasses@. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into Asubclasses@ (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 US Patent 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 Fcγ RIII 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)), and regulates homeostasis of immunoglobulins.

"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.

"Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994). HER2 antibody scFv fragments are described in WO93/16185; U.S. Patent No. 5,571,894; and U.S. Patent No. 5,587,458.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (V_H) connected to a variable light domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

A "naked antibody" is an antibody that is not conjugated to a heterologous molecule, such as a cytotoxic moiety or radiolabel.

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 "affinity matured" antibody is one with one or more alterations in one or more hypervariable regions thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks *et al. Bio/Technology* 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas *et al. Proc Nat. Acad. Sci, USA* 91:3809-3813 (1994); Schier *et al. Gene* 169:147-155 (1995); Yelton *et al. J. Immunol.* 155:1994-2004 (1995); Jackson *et al., J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al, J. Mol. Biol.* 226:889-896 (1992).

The term "main species antibody" herein refers to the antibody structure in a composition which is the quantitatively predominant antibody molecule in the composition. In one embodiment, the main species antibody is a HER2 antibody, such as an antibody that binds to Domain II of HER2, antibody that inhibits HER dimerization more effectively than trastuzumab, and/or an antibody which binds to a heterodimeric binding site of HER2. The preferred embodiment herein of the main species antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably comprising the light chain and heavy chain amino acid sequences in SEQ ID Nos. 13 and 14 (pertuzumab).

An "amino acid sequence variant" antibody herein is an antibody with an amino acid sequence which differs from a main species antibody. Ordinarily, amino acid sequence variants will possess at least about 70% homology with the main species antibody, and preferably, they will be at least about 80%, more preferably at least about 90% homologous with the main species antibody. The amino acid sequence variants possess substitutions, deletions, and/or additions at certain positions within or adjacent to the amino acid sequence of the main species antibody. Examples of amino acid sequence variants herein include an acidic variant (*e.g.* deamidated antibody variant), a basic variant, an antibody with an amino-terminal leader extension (*e.g.* VHS-) on one or two light chains thereof, an antibody with a C-terminal lysine residue on one or two heavy chains thereof, etc, and includes combinations of variations to the amino acid sequences of heavy and/or light chains. The antibody variant of particular interest herein is the antibody comprising an amino-terminal leader extension on one or two light chains thereof, optionally

further comprising other amino acid sequence and/or glycosylation differences relative to the main species antibody.

A "glycosylation variant" antibody herein is an antibody with one or more carbohydrate moeities attached thereto which differ from one or more carbohydate moieties attached to a main species antibody. Examples of glycosylation variants herein include antibody with a G1 or G2 oligosaccharide structure, instead a G0 oligosaccharide structure, attached to an Fc region thereof, antibody with one or two carbohydrate moieties attached to one or two light chains thereof, antibody with no carbohydrate attached to one or two heavy chains of the antibody, etc, and combinations of glycosylation alterations.

Where the antibody has an Fc region, an oligosaccharide structure may be attached to one or two heavy chains of the antibody, *e.g.* at residue 299 (298, Eu numbering of residues). For pertuzumab, G0 was the predominant oligosaccharide structure, with other oligosaccharide structures such as G0-F, G-1, Man5, Man6, G1-1, G1(1-6), G1(1-3) and G2 being found in lesser amounts in the pertuzumab composition.

Unless indicated otherwise, a AG1 oligosaccharide structure@ herein includes G-1, G1-1, G1(1-6) and G1(1-3) structures.

An "amino-terminal leader extension" herein refers to one or more amino acid residues of the amino-terminal leader sequence that are present at the amino-terminus of any one or more heavy or light chains of an antibody. An exemplary amino-terminal leader extension comprises or consists of three amino acid residues, VHS, present on one or both light chains of an antibody variant.

A "deamidated" antibody is one in which one or more asparagine residues thereof has been derivitized, *e.g.* to an aspartic acid, a succinimide, or an iso-aspartic acid.

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 (including medulloblastoma and retinoblastoma), sarcoma (including liposarcoma and synovial cell sarcoma), neuroendocrine tumors (including carcinoid tumors, gastrinoma, and islet cell cancer), mesothelioma, schwannoma (including acoustic neuroma), meningioma, adenocarcinoma, melanoma, 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 (SCLC), non-small cell lung cancer (NSCLC), 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 (including metastatic 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, testicular cancer, esophagael cancer, tumors of the biliary tract, as well as head and neck cancer.

An "advanced" cancer is one which has spread outside the site or organ of origin, either by local invasion or metastasis.

A "refractory" cancer is one which progresses even though an anti-tumor agent, such as a chemotherapeutic agent, is being administered to the cancer patient. An example of a refractory cancer is one which is platinum refractory.

A "recurrent" cancer is one which has regrown, either at the initial site or at a distant site, after a response to initial therapy.

Herein, a "patient" is a human patient. The patient may be a Acancer patient,@ *i.e.* one who is suffering or at risk for suffering from one or more symptoms of cancer.

A "tumor sample" herein is a sample derived from, or comprising tumor cells from, a patient=s tumor. Examples of tumor samples herein include, but are not limited to, tumor biopsies, circulating tumor cells, circulating plasma proteins, ascitic fluid, primary cell cultures or cell lines derived from tumors or exhibiting tumor-like properties, as well as preserved tumor samples, such as formalin-fixed, paraffin-embedded tumor samples or frozen tumor samples.

A "fixed" tumor sample is one which has been histologically preserved using a fixative.

A "formalin-fixed" tumor sample is one which has been preserved using formaldehyde as the fixative.

An "embedded" tumor sample is one surrounded by a firm and generally hard medium such as paraffin, wax, celloidin, or a resin. Embedding makes possible the cutting of thin sections for microscopic examination or for generation of tissue microarrays (TMAs).

A "paraffin-embedded" tumor sample is one surrounded by a purified mixture of solid hydrocarbons derived from petroleum.

Herein, a "frozen" tumor sample refers to a tumor sample which is, or has been, frozen.

A cancer or biological sample which "displays HER expression, amplification, or activation" is one which, in a diagnostic test, expresses (including overexpresses) a HER receptor, has amplified HER gene, and/or otherwise demonstrates activation or phosphorylation of a HER receptor.

A cancer or biological sample which "displays HER activation" is one which, in a diagnostic test, demonstrates activation or phosphorylation of a HER receptor. Such activation can be determined directly (*e.g.* by measuring HER phosphorylation by ELISA) or indirectly (*e.g.* by gene expression profiling or by detecting HER heterodimers, as described herein).

Herein, "gene expression profiling" refers to an evaluation of expression of one or more genes as a surrogate for determining HER phosphorylation directly.

A "phospho-ELISA assay" herein is an assay in which phosphorylation of one or more HER receptors, especially HER2, is evaluated in an enzyme-linked immunosorbent assay (ELISA) using a reagent, usually an antibody, to detect phosphorylated HER receptor, substrate, or downstream signaling molecule. Preferably, an antibody which detects phosphorylated HER2 is used. The assay may be performed on cell lysates, preferably from fresh or frozen biological samples.

A cancer cell with "HER receptor overexpression or amplification" is one which has significantly higher levels of a HER receptor protein or gene compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. HER receptor overexpression or amplification may be determined in a diagnostic or prognostic assay by evaluating increased levels of the HER protein present on the surface of a cell (*e.g.* via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of HER-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 quantitative real time PCR (qRT-PCR). One may also study HER receptor overexpression or amplification by measuring shed antigen (*e.g.*, HER extracellular domain) in a biological fluid such as serum (see, *e.g.*, U.S. Patent No. 4,933,294 issued June 12, 1990; WO91/05264 published April 18, 1991; U.S. Patent 5,401,638 issued March 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.

Conversely, a cancer which "does not overexpress or amplify HER receptor" is one which does not have higher than normal levels of HER receptor protein or gene compared to a noncancerous cell of the same tissue type. Antibodies that inhibit HER dimerization, such as pertuzumab, may be used to treat cancer which does not overexpress or amplify HER2 receptor.

Herein, an "anti-tumor agent" refers to a drug used to treat cancer. Non-limiting examples of anti-tumor agents herein include chemotherapeutic agents, HER dimerization inhibitors, HER antibodies, antibodies directed against tumor associated antigens, anti-hormonal compounds, cytokines, EGFR-targeted drugs, anti-angiogenic agents, tyrosine kinase inhibitors, growth inhibitory agents and antibodies, cytotoxic agents, antibodies that induce apoptosis, COX inhibitors, farnesyl transferase inhibitors, antibodies that binds oncofetal protein CA 125, HER2 vaccines, Raf or ras inhibitors, liposomal doxorubicin, topotecan, taxane, dual tyrosine kinase inhibitors, TLK286, EMD-7200, pertuzumab, trastuzumab, erlotinib, and bevacizumab.

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 antibody 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 (see below). Examples of HER2 antibodies that induce apoptosis are 7C2 and 7F3.

The "epitope 2C4" is the region in the extracellular domain of HER2 to which the antibody 2C4 binds. In order to screen for antibodies which bind to the 2C4 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. Preferably the antibody blocks 2C4's binding to HER2 by about 50% or more. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 2C4 epitope of HER2. Epitope 2C4 comprises residues from Domain II in the extracellular domain of HER2. 2C4 and pertuzumab binds to the extracellular domain of HER2 at the junction of domains I, II and III. Franklin *et al. Cancer Cell* 5:317-328 (2004).

The "epitope 4D5" is the region in the extracellular domain of HER2 to which the antibody 4D5 (ATCC CRL 10463) and trastuzumab bind. This epitope is close to the transmembrane domain of HER2, and within Domain IV of HER2. To screen for antibodies which bind to the 4D5 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 assess whether the antibody binds to the 4D5 epitope of HER2 (*e.g.* any one or more residues in the region from about residue 529 to about residue 625, inclusive of the HER2 ECD, residue numbering including signal peptide).

The "epitope 7C2/7F3" is the region at the N terminus, within Domain I, of the extracellular domain of HER2 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 HER2 (*e.g.* any one or more of residues in the region from about residue 22 to about residue 53 of the HER2 ECD, residue numbering including signal peptide).

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with cancer as well as those in which cancer is to be prevented. Hence, the patient to be treated herein may have been diagnosed as having cancer or may be predisposed or susceptible to cancer.

The term "effective amount" refers to an amount of a drug effective to treat cancer in the patient. The 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. The effective amount may extend progression free survival (*e.g.* as measured by Response Evaluation Criteria for Solid Tumors, RECIST, or CA-125 changes), result in an objective response (including a partial response, PR, or complete respose, CR), increase overall survival time, and/or improve one or more symptoms of cancer (*e.g.* as assessed by FOSI).

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 CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; TLK 286 (TELCYTA™); acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; 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, and ranimnustine; bisphosphonates, such as clodronate; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)) and anthracyclines such as annamycin, AD 32, alcarubicin, daunorubicin, dexrazoxane, DX-52-1, epirubicin, GPX-100, idarubicin, KRN5500, menogaril, dynemicin, including dynemicin A, an esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, liposomal doxorubicin, and deoxydoxorubicin), esorubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; folic acid analogues such as denopterin, pteropterin, and trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; anti-adrenals such as aminoglutethimide, mitotane, and trilostane; folic acid replenisher such as folinic acid (leucovorin); aceglatone; anti-folate anti-neoplastic agents such as ALIMTA®, LY231514 pemetrexed, dihydrofolate reductase inhibitors such as methotrexate, anti-metabolites such as 5-fluorouracil (5-FU) and its prodrugs such as UFT, S-1 and capecitabine, and thymidylate synthase inhibitors and glycinamide ribonucleotide formyltransferase inhibitors such as raltitrexed (TOMUDEX^{RM}, *TDX*); inhibitors of dihydropyrimidine dehydrogenase such as eniluracil; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK7 polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids and taxanes, *e.g.*, TAXOL® paclitaxel (Bristol-Myers

Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® docetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; platinum; platinum analogs or platinum-based analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine (VELBAN®); etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); vinca alkaloid; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATINTM) combined with 5-FU and leucovorin.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

An "antimetabolite chemotherapeutic agent" is an agent which is structurally similar to a metabolite, but can not be used by the body in a productive manner. Many antimetabolite chemotherapeutic agents interfere with the production of the nucleic acids, RNA and DNA. Examples of antimetabolite chemotherapeutic agents include gemcitabine (GEMZAR®), 5-fluorouracil (5-FU), capecitabine (XELODA™), 6-mercaptopurine, methotrexate, 6-thioguanine, pemetrexed, raltitrexed, arabinosylcytosine ARA-C cytarabine (CYTOSAR-U®), dacarbazine

(DTIC-DOME®), azocytosine, deoxycytosine, pyridmidene, fludarabine (FLUDARA®), cladrabine, 2-deoxy-D-glucose etc. The preferred antimetabolite chemotherapeutic agent is gemcitabine.

"Gemcitabine" or A"2'-deoxy-2', 2'-difluorocytidine monohydrochloride (b-isomer)" is a nucleoside analogue that exhibits antitumor activity. The empirical formula for gemcitabine HCl is C9H11F2N3O4 A HCl. Gemcitabine HCl is sold by Eli Lilly under the trademark GEMZAR®.

A "platinum-based chemotherapeutic agent" comprises an organic compound which contains platinum as an integral part of the molecule. Examples of platinum-based chemotherapeutic agents include carboplatin, cisplatin, and oxaliplatinum.

By "platinum-based chemotherapy" is intended therapy with one or more platinum-based chemotherapeutic agents, optionally in combination with one or more other chemotherapeutic agents.

By "chemotherapy-resistant" cancer is meant that the cancer patient has progressed while receiving a chemotherapy regimen (*i.e.* the patient is "chemotherapy refractory"), or the patient has progressed within 12 months (for instance, within 6 months) after completing a chemotherapy regimen.

By "platinum-resistant" cancer is meant that the cancer patient has progressed while receiving platinum-based chemotherapy (*i.e.* the patient is "platinum refractory"), or the patient has progressed within 12 months (for instance, within 6 months) after completing a platinum-based chemotherapy regimen.

An "anti-angiogenic agent" refers to a compound which blocks, or interferes with to some degree, the development of blood vessels. The anti-angiogenic factor may, for instance, be a small molecule or antibody that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. The preferred anti-angiogenic factor herein is an antibody that binds to vascular endothelial growth factor (VEGF), such as bevacizumab (AVASTIN®).

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin;

thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

As used herein, the term "EGFR-targeted drug" refers to a therapeutic agent that binds to EGFR and, optionally, inhibits EGFR activation. Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US Patent No. 4,943, 533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF (see WO98/50433, Abgenix); EMD 55900 (Stragliotto *et al. Eur. J. Cancer* 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF-alpha for EGFR binding; and mAb 806 or humanized mAb 806 (Johns *et al., J. Biol. Chem.* 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, *e.g.*, EP659,439A2, Merck Patent GmbH). Examples of small molecules that bind to EGFR include ZD1839 or Gefitinib (IRESSA; Astra Zeneca); CP-358774 or Erlotinib (TARCEVA™; Genentech/OSI); and AG1478, AG1571 (SU 5271; Sugen); EMD-7200.

A "tyrosine kinase inhibitor" is a molecule which inhibits tyrosine kinase activity of a tyrosine kinase such as a HER receptor. Examples of such inhibitors include the EGFR-targeted drugs noted in the preceding paragraph; small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda; CP-724,714, an oral selective inhibitor of the ErbB2 receptor tyrosine kinase (Pfizer and OSI); dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 and EGFR-overexpressing cells; GW572016 (available from Glaxo) an oral HER2 and EGFR tyrosine kinase inhibitor; PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibits Raf-1 signaling; non-HER targeted TK inhibitors such as Imatinib mesylate (Gleevac™) available

from Glaxo; MAPK extracellular regulated kinase I inhibitor CI-1040 (available from Pharmacia); quinazolines, such as PD 153035,4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines; pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lamber); antisense molecules *(e.g.* those that bind to HER-encoding nucleic acid); quinoxalines (US Patent No. 5,804,396); tryphostins (US Patent No. 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-HER inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); Imatinib mesylate (Gleevac; Novartis); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone); or as described in any of the following patent publications: US Patent No. 5,804,396; WO99/09016 (American Cyanimid); WO98/43960 (American Cyanamid); WO97/38983 (Warner Lambert); WO99/06378 (Warner Lambert); WO99/06396 (Warner Lambert); WO96/30347 (Pfizer, Inc); WO96/33978 (Zeneca); WO96/3397 (Zeneca); and WO96/33980 (Zeneca).

A "fixed " or "flat" dose of a therapeutic agent herein refers to a dose that is administered to a human patient without regard for the weight (WT) or body surface area (BSA) of the patient. The fixed or flat dose is therefore not provided as a mg/kg dose or a mg/m^2 dose, but rather as an absolute amount of the therapeutic agent.

A "loading" dose herein generally comprises an initial dose of a therapeutic agent administered to a patient, and is followed by one or more maintenance dose(s) thereof. Generally, a single loading dose is administered, but multiple loading doses are contemplated herein. Usually, the amount of loading dose(s) administered exceeds the amount of the maintenance dose(s) administered and/or the loading dose(s) are administered more frequently than the maintenance dose(s), so as to achieve the desired steady-state concentration of the therapeutic agent earlier than can be achieved with the maintenance dose(s).

A "maintenance" dose herein refers to one or more doses of a therapeutic agent administered to the patient over a treatment period. Usually, the maintenance doses are administered at spaced treatment intervals, such as approximately every week, approximately every 2 weeks, approximately every 3 weeks, or approximately every 4 weeks.

II. Production of Antibodies

Since, in the preferred embodiment, the HER dimerization inhibitor is an antibody, a description follows as to exemplary techniques for the production of HER antibodies used in accordance with the present invention. The HER antigen to be used for production of antibodies may be, *e.g.*, a soluble form of the extracellular domain of a HER receptor or a portion thereof, containing the desired epitope. Alternatively, cells expressing HER at their cell surface (*e.g.*

NIH-3T3 cells transformed to overexpress HER2; 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 HER receptor 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 1/5 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

Various methods for making monoclonal antibodies herein are available in the art. For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), by recombinant DNA methods (U.S. Patent 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, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland 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. Patent 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. Patent 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.

US Patent No. 6,949,245 describes production of exemplary humanized HER2 antibodies which bind HER2 and block ligand activation of a HER receptor. The humanized antibody of particular interest herein blocks EGF, TGF-α and/or HRG mediated activation of MAPK essentially as effectively as murine monoclonal antibody 2C4 (or a Fab fragment thereof) and/or binds HER2 essentially as effectively as murine monoclonal antibody 2C4 (or a Fab fragment thereof). 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.

An exemplary humanized antibody of interest herein comprises variable heavy domain complementarity determining residues GFTFTDYTMX, where X is preferrably D or S (SEQ ID NO:7); DVNPNSGGSIYNQRFKG (SEQ ID NO:8); and/or NLGPSFYFDY (SEQ ID NO:9), optionally comprising amino acid modifications of those CDR residues, *e.g.* where the modifications essentially maintain or improve affinity of the antibody. For example, the antibody variant of interest may have from about one to about seven or about five amino acid substitutions in the above variable heavy CDR sequences. Such antibody variants may be prepared by affinity maturation, *e.g.*, as described below. The most preferred humanized antibody comprises the variable heavy domain amino acid sequence in SEQ ID NO:4.

The humanized antibody may comprise variable light domain complementarity determining residues KASQDVSIGVA (SEQ ID NO:10); SASYX^{1}X^{2}X^3, where X^1 is preferably R or L, X^2 is preferably Y or E, and X^3 is preferably T or S (SEQ ID NO:11); and/or QQYYIYPYT (SEQ ID NO:12), *e.g.* in addition to those variable heavy domain CDR residues in the preceding paragraph. Such humanized antibodies optionally comprise amino acid modifications of the above CDR residues, *e.g.* where the modifications essentially maintain or improve affinity of the antibody. For example, the antibody variant of interest may have from about one to about seven or about five amino acid substitutions in the above variable light CDR sequences. Such antibody variants may be prepared by affinity maturation, *e.g.*, as described below. The most preferred humanized antibody comprises the variable light domain amino acid sequence in SEQ ID NO:3.

The present application also contemplates affinity matured antibodies which bind HER2 and block ligand activation of a HER receptor. The parent antibody may be a human antibody or a humanized antibody, *e.g.*, one comprising the variable light and/or variable heavy sequences of SEQ ID Nos. 3 and 4, respectively (*i.e.* comprising the VL and/or VH of pertuzumab). The affinity matured antibody preferably binds to HER2 receptor with an affinity superior to that of murine 2C4 or pertuzumab (*e.g.* from about two or about four fold, to about 100 fold or about 1000 fold improved affinity, *e.g.* as assessed using a HER2-extracellular domain (ECD) ELISA) . Exemplary variable heavy CDR residues for substitution include H28, H30, H34, H35, H64, H96, H99, or combinations of two or more (*e.g.* two, three, four, five, six, or seven of these residues). Examples of variable light CDR residues for alteration include L28, L50, L53, L56, L91, L92, L93, L94, L96, L97 or combinations of two or more (*e.g.* two to three, four, five or up to about ten of these residues).

Various forms of the humanized antibody or affinity matured antibody are contemplated. For example, the humanized antibody or affinity matured antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody or affinity matured antibody may be an intact antibody, such as an intact IgG1 antibody. The preferred intact IgG1 antibody comprises the light chain sequence in SEQ ID NO:13 and the heavy chain sequence in SEQ ID NO:14.

(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. Patent 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. Patent 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. Patents 5,567,610 and 5,229,275).

Human HER2 antibodies are described in U.S. Patent No. 5,772,997 issued June 30, 1998 and WO 97/00271 published January 3, 1997.

(v) Antibody fragments

Various techniques have been developed for the production of antibody fragments comprising one or more antigen binding regions. 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. Patent No. 5,571,894; and U.S. Patent No. 5,587,458. The antibody fragment may also be a Alinear antibody@, *e.g.*, as described in U.S. Patent 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 HER2 protein. Other such antibodies may combine a HER2 binding site with binding site(s) for EGFR, HER3 and/or HER4. Alternatively, a HER2 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 HER2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express HER2. These antibodies possess a HER2-binding arm and an arm which binds the cytotoxic agent (*e.g.* saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* $F(ab')_2$ bispecific antibodies).

WO 96/16673 describes a bispecific HER2/FcγRIII antibody and U.S. Patent No. 5,837,234 discloses a bispecific HER2/FcγRI antibody IDM1 (Osidem). A bispecific HER2/Fcα antibody is shown in WO98/02463. U.S. Patent No. 5,821,337 teaches a bispecific HER2/CD3 antibody. MDX-210 is a bispecific HER2-FcγRIII Ab.

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. Patent 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.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies

have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

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 HER2 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 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 antibody are prepared by introducing appropriate nucleotide changes into the 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 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 antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the 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 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 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 antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the 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 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 I, 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)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; 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. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) acidic: Asp (D), Glu (E)

(4) basic: Lys (K), Arg (R), His(H)

Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(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 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 HER2. 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.

Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

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.

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).

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 A1, Presta, L. See also US 2004/0093621 A1 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO03/011878, Jean-Mairet *et al.* and US Patent No. 6,602,684, Umana *et al.* Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO97/30087, Patel *et al.* See, also, WO98/58964 (Raju, S.) and WO99/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof.

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).

WO00/42072 (Presta, L.) describes antibodies with improved ADCC function in the presence of human effector cells, where the antibodies comprise amino acid substitutions in the Fc region thereof. Preferably, the antibody with improved ADCC comprises substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Preferably the altered Fc region is a human IgG1 Fc region comprising or consisting of substitutions at one, two or three of these positions. Such substitutions are optionally combined with substitution(s) which increase C1q binding and/or CDC.

Antibodies with altered C1q binding and/or complement dependent cytotoxicity (CDC) are described in WO99/51642, US Patent No. 6,194,551B1, US Patent No. 6,242,195B1, US Patent No. 6,528,624B1 and US Patent No. 6,538,124 (Idusogie *et al.*). The antibodies comprise an amino acid substitution at one or more of amino acid positions 270, 322, 326, 327, 329, 313, 333 and/or 334 of the Fc region thereof (Eu numbering of residues).

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 US Patent 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.

Antibodies with improved binding to the neonatal Fc receptor (FcRn), and increased half-lives, are described in WO00/42072 (Presta, L.) and US2005/0014934A1 (Hinton *et al.*). These antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. For example, the Fc region may have substitutions at one or more of positions 238, 250, 256, 265, 272, 286, 303, 305, 307, 311, 312, 314, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424, 428 or 434 (Eu numbering of residues). The preferred Fc region-comprising antibody variant with improved FcRn binding comprises amino acid substitutions at one, two or three of positions 307, 380 and 434 of the Fc region thereof (Eu numbering of residues).

Engineered antibodies with three or more (preferably four) functional antigen binding sites are also contemplated (US Appln No. US2002/0004587 A1, Miller *et al.*).

Nucleic acid molecules encoding amino acid sequence variants of the 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 antibody.

(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.

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

For example, inhibition of HRG binding to MCF7 breast tumor cell lines by HER2 antibodies may be performed using monolayer MCF7 cultures on ice in a 24-well-plate format essentially as described in US Patent No. 6,949,245. HER2 monoclonal antibodies may be added to each well and incubated for 30 minutes. 125I-labeled rHRGβ1177-224 (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 IC50 value may be calculated for the antibody of interest. In one embodiment, the antibody which blocks ligand activation of a HER receptor will have an IC50 for inhibiting HRG binding to MCF7 cells in this assay of about 50nM or less, more preferably 10nM or less. Where the antibody is an antibody fragment such as a Fab fragment, the IC50 for inhibiting HRG binding to MCF7 cells in this assay may, for example, be about 100nM or less, more preferably 50nM or less.

Alternatively, or additionally, the ability of an antibody to block HER ligand-stimulated tyrosine phosphorylation of a HER receptor present in a HER hetero-oligomer may be assessed. For example, cells endogenously expressing the HER receptors or transfected to expressed them may be incubated with the antibody and then assayed for HER 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. Patent No. 5,766,863 is also available for determining HER 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 essentially as described in US Patent No. 6,949,245. For example, the MCF7 cells may be plated in 24-well plates and monoclonal antibodies to HER2 may be added to each well and incubated for 30 minutes at room temperature; then $rHRG\beta1_{177-244}$ 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 I 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 a HER receptor will have an IC_{50} for inhibiting HRG stimulation of p180 tyrosine phosphorylation in this assay of about 50nM or less, more preferably 10nM 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 100nM or less, more preferably 50nM 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 be treated with a HER2 monoclonal antibody (10µg/mL) for 4 days and stained with crystal violet. Incubation with a HER2 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 HER2 antibody of interest may block heregulin dependent association of HER2 with HER3 in both MCF7 and SK-BR-3 cells as determined in a co-immunoprecipitation experiment such as that described in US Patent No. 6,949,245 substantially more effectively than monoclonal antibody 4D5, and preferably substantially more effectively than monoclonal antibody 7F3.

To identify growth inhibitory HER2 antibodies, one may screen for antibodies which inhibit the growth of cancer cells which overexpress HER2. 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. Patent 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 35mm cell culture dish (2mls/35mm dish). 0.5 to 30 µg/ml of the HER2 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. See US Pat No. 5,677,171 for assays for screening for growth inhibitory antibodies, such as 4D5 and 3E8.

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 10μg/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) (1μg/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 9μg/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. See WO98/17797 for assays for screening for antibodies which induce apoptosis, such as 7C2 and 7F3.

To screen for antibodies which bind to an epitope on HER2 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 to assess whether the antibody cross-blocks binding of an antibody, such as 2C4 or pertuzumab, to HER2. Alternatively, or additionally, epitope mapping can be performed by methods known in the art and/or one can study the antibody-HER2 structure (Franklin *et al. Cancer Cell* 5:317-328 (2004)) to see what domain(s) of HER2 is/are bound by the antibody.

(ix) Pertuzumab compositions

In one embodiment of a HER2 antibody composition, the composition comprises a mixture of a main species pertuzumab antibody and one or more variants thereof. The preferred embodiment herein of a pertuzumab main species antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably

comprising a light chain amino acid sequence selected from SEQ ID No. 13 and 17, and a heavy chain amino acid sequence selected from SEQ ID No. 14 and 18 (including deamidated and/or oxidized variants of those sequences). In one embodiment, the composition comprises a mixture of the main species pertuzumab antibody and an amino acid sequence variant thereof comprising an amino-terminal leader extension. Preferably, the amino-terminal leader extension is on a light chain of the antibody variant (*e.g.* on one or two light chains of the antibody variant). The main species HER2 antibody or the antibody variant may be an full length antibody or antibody fragment (*e.g.* Fab of F(ab=)2 fragments), but preferably both are full length antibodies. The antibody variant herein may comprise an amino-terminal leader extension on any one or more of the heavy or light chains thereof. Preferably, the amino-terminal leader extension is on one or two light chains of the antibody. The amino-terminal leader extension preferably comprises or consists of VHS-. Presence of the amino-terminal leader extension in the composition can be detected by various analytical techniques including, but not limited to, N-terminal sequence analysis, assay for charge heterogeneity (for instance, cation exchange chromatography or capillary zone electrophoresis), mass spectrometry, etc. The amount of the antibody variant in the composition generally ranges from an amount that constitutes the detection limit of any assay (preferably N-terminal sequence analysis) used to detect the variant to an amount less than the amount of the main species antibody. Generally, about 20% or less (*e.g.* from about 1% to about 15%, for instance from 5% to about 15%) of the antibody molecules in the composition comprise an amino-terminal leader extension. Such percentage amounts are preferably determined using quantitative N-terminal sequence analysis or cation exchange analysis (preferably using a high-resolution, weak cation-exchange column, such as a PROPAC WCX-10™ cation exchange column). Aside from the amino-terminal leader extension variant, further amino acid sequence alterations of the main species antibody and/or variant are contemplated, including but not limited to an antibody comprising a C-terminal lysine residue on one or both heavy chains thereof, a deamidated antibody variant, etc.

Moreover, the main species antibody or variant may further comprise glycosylation variations, non-limiting examples of which include antibody comprising a G1 or G2 oligosaccharide structure attached to the Fc region thereof, antibody comprising a carbohydrate moiety attached to a light chain thereof (*e.g.* one or two carbohydrate moieties, such as glucose or galactose, attached to one or two light chains of the antibody, for instance attached to one or more lysine residues), antibody comprising one or two non-glycosylated heavy chains, or antibody comprising a sialidated oligosaccharide attached to one or two heavy chains thereof etc.

The composition may be recovered from a genetically engineered cell line, *e.g.* a Chinese Hamster Ovary (CHO) cell line expressing the HER2 antibody, or may be prepared by peptide synthesis.

(x) Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* a small molecule toxin or an enzymatically active toxin of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, a maytansine (U.S. Patent No. 5,208,020), a trichothene, and CC1065 are also contemplated herein.

In one preferred embodiment of the invention, the antibody is conjugated to one or more maytansine molecules (*e.g.* about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antibody (Chari *et al. Cancer Research* 52: 127-131 (1992)) to generate a maytansinoid-antibody immunoconjugate.

Another immunoconjugate of interest comprises an antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I; (Hinman *et al. Cancer Research* 53: 3336-3342 (1993) and Lode *et al. Cancer Research* 58: 2925-2928 (1998)). See, also, US Patent Nos. 5,714,586; 5,712,374; 5,264,586; and 5,773,001 expressly incorporated herein by reference.

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

The present invention further contemplates an immunoconjugate formed between an antibody and a compound with nucleolytic activity (*e.g.* a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

A variety of radioactive isotopes are available for the production of radioconjugated HER2 antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) 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 tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a Acleavable linker® facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari *et al. Cancer Research* 52: 127-131 (1992)) may be used.

Alternatively, a fusion protein comprising the antibody and cytotoxic agent may be made, *e.g.* by recombinant techniques or peptide synthesis.

Other immunoconjugates are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may 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, Oslo, A., Ed., (1980).

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al., Proc. Natl Acad. Sci. USA*, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon *et al. J. National Cancer Inst.*81(19)1484 (1989).

III. Selecting patients for therapy

The patient herein is subjected to a diagnostic test prior to therapy. Generally, if a diagnostic test is performed, a sample may be obtained from a patient in need of therapy. Where the subject has cancer, the sample may be a tumor sample, or other biological sample, such as a biological fluid, including, without limitation, blood, urine, saliva, ascites fluid, or derivatives such as blood serum and blood plasma, and the like.

Where the diagnostic assay is performed on a tumor sample, the tumor sample may be from an ovarian cancer, peritoneal cancer, fallopian tube cancer, metastatic breast cancer (MBC), non-small cell lung cancer (NSCLC), prostate cancer, or colorectal cancer tumor sample, etc. The biological sample herein may be a fixed sample, *e.g.* a formalin fixed, paraffin-embedded (FFPE) sample, or a frozen sample.

In one embodiment, the level of EGF and/or TGF-alpha in the patient is evaluated, wherein an elevated level thereof compared to normal levels indicates that the patient is a candidate for therapy with a HER dimerization inhibitor. Such levels of EGF and/or TGF-alpha may be assessed *in vivo* or in various biological samples taken from the patient. Preferably however, the biological sample tested is a serum or plasma sample.

Various methods for determining expression of mRNA or protein include, but are not limited to, gene expression profiling, polymerase chain reaction (PCR) including quantitative real time PCR (qRT-PCR), microarray analysis, serial analysis of gene expression (SAGE), MassARRAY, Gene Expression Analysis by Massively Parallel Signature Sequencing (MPSS), proteomics, immunohistochemistry (IHC), etc. Preferably mRNA is quantified. Such mRNA analysis is preferably performed using the technique of polymerase chain reaction (PCR), or by microarray analysis. Where PCR is employed, a preferred form of PCR is quantitative real time PCR (qRT-PCR). In one embodiment, expression of one or more of the above noted genes is deemed positive expression if it is at the median or above, *e.g.* compared to other samples of the same tumor-type. The median expression level can be determined essentially contemporaneously with measuring gene expression, or may have been determined previously.

The steps of a representative protocol for profiling gene expression using fixed, paraffin-embedded tissues as the RNA source, including mRNA isolation, purification, primer extension and amplification are given in various published journal articles (for example: Godfrey *et al. J. Molec. Diagnostics* 2: 84-91 (2000); Specht *et al., Am. J. Pathol.* 158: 419-29 (2001)). Briefly, a representative process starts with cutting about 10 microgram thick sections of paraffin-embedded tumor tissue samples. The RNA is then extracted, and protein and DNA are removed. After analysis of the RNA concentration, RNA repair and/or amplification steps may be included, if necessary, and RNA is reverse transcribed using gene specific promoters followed by PCR.

Finally, the data are analyzed to identify the best treatment option(s) available to the patient on the basis of the characteristic gene expression pattern identified in the tumor sample examined.

A specific serum ELISA bioassay protocol is provided in Example 1.

EGF and/or TGF-alpha may also be evaluated using an *in vivo* diagnostic assay, *e.g.* by administering a molecule (such as an antibody) which binds the molecule to be detected and is tagged with a detectable label (*e.g.* a radioactive isotope) and externally scanning the patient for localization of the label.

Aside from evaluation of EGF and/or TGF-alpha, one may determine HER expression or amplification in the cancer. Various diagnostic/prognostic assays are available for this. In one embodiment, HER 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 HER2 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 HER2 overexpression assessment may be characterized as not overexpressing HER2, whereas those tumors with 2+ or 3+ scores may be characterized as overexpressing HER2.

Tumors overexpressing HER2 may be 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)).

Alternatively, or additionally, FISH assays such as the INFORM™ (sold by Ventana, Arizona) or PATHVISION™ (Vysis, Illinois) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of HER2 amplification in the tumor.

In one embodiment, the cancer will be one which expresses (and may overexpress) EGFR, such expression may be evaluated as for the methods for evaluating HER2 expression as noted above.

IV. Pharmaceutical Formulations

Therapeutic formulations of the HER dimerization inhibitors 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)), generally in the form of lyophilized formulations or aqueous solutions. Antibody crystals are also contemplated (see US Pat Appln 2002/0136719). 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). Lyophilized antibody formulations are described in WO 97/04801, expressly incorporated herein by reference.

The preferred pertuzumab formulation for therapeutic use comprises 30mg/mL pertuzumab in 20mM histidine acetate, 120mM sucrose, 0.02% polysorbate 20, at pH 6.0. An alternate pertuzumab formulation comprises 25 mg/mL pertuzumab, 10 mM histidine-HCl buffer, 240 mM sucrose, 0.02% polysorbate 20, pH 6.0.

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. Various drugs which can be combined with the HER dimerization inhibitor are described in the Method Section below. 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.

V. Treatment with HER dimerization inhibitors

The invention herein provides a method for extending survival in a cancer patient who produces an elevated level of EGF and/or TGF-alpha, comprising administering a HER dimerization inhibitor to the patient in an amount which extends the patient's survival. Preferably, the HER dimerization inhibitor is a HER2 dimerization inhibitor and/or inhibits HER heterodimerization.

Methods for identify candidate patients for therapy with a HER dimerization inhibitor have been discussed in Section III above.

Examples of various cancers that can be treated with a HER dimerization inhibitor are listed in the definition section above. Preferred cancer indications include ovarian cancer; peritoneal cancer; fallopian tube cancer; breast cancer, including metastatic breast cancer (MBC); lung cancer, including non-small cell lung cancer (NSCLC); prostate cancer; and colorectal cancer. In one embodiment, the cancer which is treated is advanced, refractory, recurrent, chemotherapy-resistant, and/or platinum-resistant cancer.

Therapy with the HER dimerization inhibitor extends TTP and/or survival. In one embodiment, therapy with the HER dimerization inhibitor extends TTP or survival at least about 5%, or at least 10%, or at least 15% or at least 20%, or at least 25% more than TTP or survival achieved by administering an approved anti-tumor agent, or standard of care, for the cancer being treated.

In the preferred embodiment, the invention provides a method for extending time to disease progression (TTP) or survival in a patient with ovarian, peritoneal, or fallopian tube cancer, whose cancer displays HER2 activation, comprising administering pertuzumab to the patient in an amount which extends the patient's TTP or survival. The patient may have advanced, refractory, recurrent, chemotherapy-resistant, and/or platinum-resistant ovarian, peritoneal or fallopian tube cancer. Administration of pertuzumab to the patient may, for example, extend TTP or survival at least about 5%, or at least 10%, or at least 15%, or at least 20%, or at least 25% more than TTP or survival achieved by administering topotecan or liposomal doxorubicin to such a patient.

The HER dimerization inhibitor is administered 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 administration of the antibody is preferred.

For the prevention or treatment of cancer, the dose of HER dimerization inhibitor will depend on the type of cancer to be treated, as defined above, the severity and course of the cancer, 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.

In one embodiment, a fixed dose of HER dimerization inhibitor is administered. The fixed dose may suitably be administered to the patient at one time or over a series of treatments. Where a fixed dose is administered, preferably it is in the range from about 20mg to about 2000 mg of the HER dimerization inhibitor. For example, the fixed dose may be approximately 420mg, approximately 525mg, approximately 840mg, or approximately 1050mg of the HER dimerization inhibitor, such as pertuzumab.

Where a series of doses are administered, these may, for example, be administered approximately every week, approximately every 2 weeks, approximately every 3 weeks, or approximately every 4 weeks, but preferably approximately every 3 weeks. The fixed doses may, for example, continue to be administered until disease progression, adverse event, or other time as determined by the physician. For example, from about two, three, or four, up to about 17 or more fixed doses may be administered.

In one embodiment, one or more loading dose(s) of the antibody are administered, followed by one or more maintenance dose(s) of the antibody. In another embodiment, a plurality of the same dose are administered to the patient.

According to one preferred embodiment of the invention, a fixed dose of HER dimerization inhibitor (*e.g.* pertuzumab) of approximately 840mg (loading dose) is administered,

followed by one or more doses of approximately 420mg (maintenance dose(s)) of the antibody. The maintenance doses are preferably administered about every 3 weeks, for a total of at least two doses, up to 17 or more doses.

According to another preferred embodiment of the invention, one or more fixed dose(s) of approximately 1050mg of the HER dimerization inhibitor (*e.g.* pertzumab) are administered, for example every 3 weeks. According to this embodiment, one, two or more of the fixed doses are administered, *e.g.* for up to one year (17 cycles), and longer as desired.

In another embodiment, a fixed dose of approximately 1050mg of the HER dimerization inhibitor (*e.g.* pertuzumab) is administered as a loading dose, followed by one or more maintenance dose(s) of approximately 525mg. About one, two or more maintenance doses may be administered to the patient every 3 weeks according to this embodiment.

While the HER dimerization inhibitor may be administered as a single anti-tumor agent, the patient is optionally treated with a combination of the HER dimerization inhibitor, and one or more chemotherapeutic agent(s). Preferably at least one of the chemotherapeutic agents is an antimetabolite chemotherapeutic agent such as gemcitabine. The combined administration includes coadministration or concurrent administration, 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. Thus, the antimetabolite chemotherapeutic agent may be administered prior to, or following, administration of the HER dimerization inhibitor. In this embodiment, the timing between at least one administration of the antimetabolite chemotherapeutic agent and at least one administration of the HER dimerization inhibitor is preferably approximately 1 month or less, and most preferably approximately 2 weeks or less. Alternatively, the antimetabolite chemotherapeutic agent and the HER dimerization inhibitor are administered concurrently to the patient, in a single formulation or separate formulations. Treatment with the combination of the chemotherapeutic agent (*e.g.* antimetabolite chemotherapeutic agent such as gemcitabine) and the HER dimerization inhibitor (*e.g.* pertuzumab) may result in a synergistic, or greater than additive, therapeutic benefit to the patient.

An antimetabolite chemotherapeutic agent, if administered, is usually administered at dosages known therefor, or optionally lowered due to combined action of the drugs or negative side effects attributable to administration of the antimetabolite chemotherapeutic agent. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Where the antimetabolite chemotherapeutic agent is gemcitabine, preferably, it is administered at a dose between about 600mg/m^2 to 1250mg/m^2 (for example approximately 1000mg/m^2), for instance, on days 1 and 8 of a 3-week cycle.

Aside from the HER dimerization inhibitor and antimetabolite chemotherapeutic agent, other therapeutic regimens may be combined therewith. For example, a second (third, fourth, etc) chemotherapeutic agent(s) may be administered, wherein the second chemotherapeutic agent is either another, different antimetabolite chemotherapeutic agent, or a chemotherapeutic agent that is not an antimetabolite. For example, the second chemotherapeutic agent may be a taxane (such as paclitaxel or docetaxel), capecitabine, or platinum-based chemotherapeutic agent (such as carboplatin, cisplatin, or oxaliplatin), anthracycline (such as doxorubicin, including, liposomal doxorubicin), topotecan, pemetrexed, vinca alkaloid (such as vinorelbine), and TLK 286. ACocktails® of different chemotherapeutic agents may be administered.

Other therapeutic agents that may be combined with the HER dimerization inhibitor include any one or more of: a second, different HER dimerization inhibitor (for example, a growth inhibitory HER2 antibody such as trastuzumab, or a HER2 antibody which induces apoptosis of a HER2-overexpressing cell, such as 7C2, 7F3 or humanized variants thereof); an antibody directed against a different tumor associated antigen, such as EGFR, HER3, HER4; anti-hormonal compound, *e.g.*, an anti-estrogen compound such as tamoxifen, or an aromatase inhibitor; a cardioprotectant (to prevent or reduce any myocardial dysfunction associated with the therapy); a cytokine; an EGFR-targeted drug (such as TARCEVA®, IRESSA® or cetuximab); an anti-angiogenic agent (especially bevacizumab sold by Genentech under the trademark AVASTIN™); a tyrosine kinase inhibitor; a COX inhibitor (for instance a COX-1 or COX-2 inhibitor); non-steroidal anti-inflammatory drug, celecoxib (CELEBREX®); farnesyl transferase inhibitor (for example, Tipifarnib/ZARNESTRA™ R115777 available from Johnson and Johnson or Lonafarnib SCH66336 available from Schering-Plough); antibody that binds oncofetal protein CA 125 such as Oregovomab (MoAb B43.13); HER2 vaccine (such as HER2 AutoVac vaccine from Pharmexia, or APC8024 protein vaccine from Dendreon, or HER2 peptide vaccine from GSK/Corixa); another HER targeting therapy (*e.g.* trastuzumab, cetuximab, ABX-EGF, EMD7200, gefitinib, erlotinib, CP724714, CI1033, GW572016, IMC-11F8, TAK165, etc); Raf and/or ras inhibitor (see, for example, WO 2003/86467); doxorubicin HCl liposome injection (DOXIL®); topoisomerase I inhibitor such as topotecan; taxane; HER2 and EGFR dual tyrosine kinase inhibitor such as lapatinib/GW572016; TLK286 (TELCYTA®); EMD-7200; a medicament that treats nausea such as a serotonin antagonist, steroid, or benzodiazepine; a medicament that prevents or treats skin rash or standard acne therapies, including topical or oral antibiotic; a medicament that treats or prevents diarrhea; a body temperature-reducing medicament such as acetaminophen, diphenhydramine, or meperidine; hematopoietic growth factor, etc.

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 HER dimerization inhibitor.

In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

Where the inhibitor is an antibody, preferably the administered antibody is a naked antibody. However, the inhibitor administered may be conjugated with a cytotoxic agent. Preferably, the conjugated inhibitor and/or antigen to which it is bound is/are internalized by the cell, resulting in increased therapeutic efficacy of the conjugate in killing the cancer cell to which it binds. In a preferred embodiment, the cytotoxic agent targets or interferes with nucleic acid in the cancer cell. Examples of such cytotoxic agents include maytansinoids, calicheamicins, ribonucleases and DNA endonucleases.

The present application contemplates administration of the HER dimerization inhibitor by gene therapy. See, for example, WO96/07321 published March 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells; *in vivo* and *ex vivo*. For *in vivo* delivery the nucleic acid is injected directly into the patient, usually at the site where the antibody is required. For *ex vivo* treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes which are implanted into the patient (see, *e.g.* U.S. Patent Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. A commonly used vector for *ex vivo* delivery of the gene is a retrovirus.

The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins

that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al., J. Biol. Chem.* 262:4429-4432 (1987); and Wagner *et al., Proc. Natl. Acad. Sci. USA* 87:3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols see Anderson *et al., Science* 256:808-813 (1992). See also WO 93/25673 and the references cited therein.

VI. Deposit of Materials

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

These deposits were 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 a viable culture of the deposit for 30 years from the date of deposit. The deposits 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 that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of the pertinent U.S. patent, assures permanent and unrestricted availability of the progeny of the culture of the deposit 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 § 1.14 with particular reference to 886 OG 638).

Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLE 1

CLINICAL SERUM BIOMARKER ANALYSIS IN PATIENTS WITH OVARIAN CANCER TREATED WITH PERTUZUMAB

Study Design

A Phase II, open-label, single-arm, multicenter study was performed to evaluate the effect of tumor-based HER2 activation of the efficacy of rhuMAb 2C4 (pertuzumab) in subjects with advanced, refractory or recurrent ovarian cancer.

In Cohort 1 of the trial, 65 subjects with advanced ovarian cancer that was refractory to, or has recurred following, prior chemotherapy, were enrolled, and were to receive 420 mg per cycle rhuMAb (pertuzumab). Of these, 61 subjects were treated, 4 subjects withdrew from the study and did not receive any treatment with pertuzumab.

Subjects who enrolled in Cohort 1 and fulfilled the eligibility criteria, underwent a biopsy of tumor tissue or aspiration of tumor cells from ascitic fluid. This tissue was analyzed for HER2 phosphorylation by ELISA, quantitatively measuring phosphorylated HER2 and total HER2 in the samples.

Pertuzumab was provided as a single-use formulation containing 25 mg/mL rhuMAb 2C4 formulated in 10 mM L-histidine (pH 6.0), 240 mM sucrose, and 0.02% polysorbate 20. Each 10-cc vial contained about 175 mg of rhuMAb 2C4 (7.0 mL/vial). Upoon receipt, vials were refrigerated at 2°C-8°C until use. Because the formulation does not contain a preservative, inctruction were give to puncture the vial seal only once. Any remaining solution was discarded. The solution of rhuMAb 2C4 for infusion diluted in PVC polyethylene and non-PVC polyolefin bags containing 0.9% Sodium Chloride Injection, USP, was allowed to be stored at 2°C-8°C for 24 hours prior to use.

Pertuzumab was administered as an IV infusion every 3 weeks for up to one year (17 cycles) for subjects who showed no evidence of progressive disease. Subjects decived a loading dose of 840 m (Cycle 1) followed by 420 mg in Cycle 2 and beyond.

After enrollment in Cohort 1 was completed, enrollment in Cohort 2 commenced. Subjects in Cohort 2, who fulfill the eligibility criteria, receive 1050 mg pertuzumab, administered as an IV infusion every 3 weeks for up to one year (17 cycles). Subjects in Cohort 2 (which is ongoing) do not undergo a biopsy of tumor tissue or aspiration of tumor cells from ascites fluids.

Response has been assessed after 6 weeks, 3 months, and every 3 months thereafter. An additional response is assessed at 18 weeks (4.5 months) for subjects in Cohort 2 only.

Measurable disease has been assessed using the Response Evaluation Criteria for Solid Tumors (RECIST) (see, for example, Therasse *et al., J. Nat. Cancer Inst.* 92(3): 205-216 (2000)), by clinical evaluation and CT scan or equivalent. Response for subjects with evaluable but no measurable disease has assessed according to changes in CA-125 and clinical and rediologic evidence of disease.

Primary efficacy endpoint:

Best overall response at any time during the study following initiation of treatment with pertuzumab, as determined by investigator assessment using RECIST or by CA-125 changes, following initiation of treatment with pertuzumab.

Secondary efficacy endpoints:

Time To Disease Progression (TTDP),

Duration of response,

survival time (Overall Survival, OS), and

percentage of subjects free from disease progression at 3, 6, and 12 months (Disease-Free Survival, DFS).

Disease progression was defined as documented progressive disease or death, whichever occurred first.

Time To Disease Progression (TTDP) was defined as the time from the first day of study drug treatment (Day 1) until the time of documented disease progression or death.

Duration of survival was defined as the time from Day 1 until the time of death.

Duration of response was defined as the time from the initial complete or partial response to the time of disease progression or death.

The 95% exact confidence interval was constructed for percentage of the subjects free from progression after 3, 6, and 12 months in the study.

Median time to disease progression and duration of survival were calculated using Kaplan-Meier survival methods.

Exploratory assessment of biologic markers was incorporated into this trial. The purpose of this assessment was to find one or more pre-treatment biological markers that may predict which subjects will, or will not, respond to pertuzumab treatment, or to identify one or more post-therapeutic biologic markers that might act as a biomarker of pertuzumab activity. In particular, assessment of biological markers allows identification of a patient population that is especially likely to benefit from pertuzumab treatment, as measured by one or more significant end points, such as overall survival (OS), or disease-free survival (DFS).

Thus gene expression profiling has been performed on normal ovarian epithelial tissue and ovarian epithelial tumors. Ovarian tumor samples obtained in this study were subjected to RNA expression profiling in order to explore the relationship between RNA expression and response to pertuzumab.

Measurement of Serum Biomarkers

Blood sera from HER2 expressing metastatic breast cancer patients treated with pertuzumab were assessed for levels of amphiregulin, EGF, TGF-alpha and shedded HER2 (HER2 ECD), as described below.

Kits used for assessment of the serum biomarkers:

Marker	Assay	Distribution
HER2-ECD	Bayer HER-2/neu ELISA, Cat.#: EL501	DakoCytomation N.V./S.A.,Interleuvenlaan 12B, B-3001 Heverlee
Amphiregulin	DuoSet ELISA Development System Human Amphiregulin, Cat. #: DY262	R&D Systems Ltd., 19 Barton Lane, Abingdon OX14 3NB, UK
EGF	Quantikine human EGF ELISA kit, Cat. #: DEG00	R&D Systems Ltd., 19 Barton Lane, Abingdon OX14 3NB, UK
TGF-alpha	Quantikine® Human TGF-alpha Immunoassay, Cat. #: DTGA00	R&D Systems Ltd., 19 Barton Lane, Abingdon OX14 3NB, UK

Protocols

HER2-ECD

HER2-ECD ELISA was performed according to the recommendations of the manufacturer.

Amphiregulin

Reagents, standard dilutions and samples were prepared following the manufacturer's instructions. EvenCoat Goat Anti-mouse IgG microplate strips (R&D, Cat. # CP002; not provided with the kit) were attached to the plate to create an ELISA plate. 100 µl diluted capture antibody (provided with the kit; 1:180 in PBS) were added to each well, and the wells were incubated at room temperature for one hour.

Each well was aspirated and washed, and the process was repeated three times for a total of four washes. The wells were washed by filling each well with 400 µl Wash Buffer (0.05 % Tween-20 in PBS), using a manifold dispenser, and subsequent aspiration. After the last wash, any remaining Wash Buffer was removed by aspiration. The plate was then inverted and blotted against clean paper towels.

100 µl standard dilution or diluted sample (see below) per well were added. Tip was changed after every pipetting step. The plate was covered with the adhesive strip (provided with the kit) and incubated for 2 hours at room temperature on a rocking platform. Thereafter, the aspiration and wash steps were repeated as described above.

The aspirated samples and wash solutions were treated with laboratory disinfectant.

100 µl Detection Antibody (provided with the kit) was added, diluted 1:180 in Reagent

Diluent (1 % BSA, Roth; Albumin Fraction V, Cat. # T844.2, in PBS) per well, and the plate was incubated for 2 hours at room temperature. Thereafter, the aspiration and wash steps were repeated as described above.

100 µl working dilution of the Streptavidin-HRP were added to each well (provided with the kit; 1:200 dilution in Reagent diluent), and the wells were covered with a new adhesive strip and incubated for 20 minutes at room temperature. The aspiration and wash steps were repeated as described above.

Add 100 µl Substrate Solution (R&D, Cat. # DY999; not provided with the kit) were added to each well, and the wells were incubated at room temperature for 20 minutes, under protection from light.

50 µl Stop Solution (1.5 M H_2SO_4 (Schwefelsäure reinst, Merck, Cat. # 713)) were added to each well, followed by careful mixing. The optical density of each well was determined immediately, using a microplate reader set to 450 nm.

Amphiregulin standard curve:

A 40 ng/ml amphiregulin stock solution was prepared in 1 % BSA in PBS, aliquotted and stored at–80 °C. Amphiregulin solutions in 20 % BSA in PBS were not stable beyond 2 weeks and were therefore not used. From the aliquotted amphiregulin stock solution, the amphiregulin standard curve was prepared freshly in 20 % BSA in PBS prior to each experiment. The highest concentration was 1000 pg/ml (1:40 dilution of the amphiregulin stock solution). The standards provided with the ELISA kit produced a linear standard curve. Excel-based analysis of the curves allowed the determination of curve equations for every ELISA.

Amphiregulin samples:

When samples were diluted 1:1 in Reagent Diluent, all samples were within the linear range of the ELISA. Each sample was measured in duplicates. Dependent on the quality of the data, and on sufficient amounts of serum, determinations were repeated in subsequent experiments if necessary.

EGF

Reagents, standard dilutions and samples were prepared following the manufacturer's instructions. Excess antibody-coated microtiter plate strips (provided with the kit) were removed from the frame to create an ELISA plate. After determining the required number of wells and the plate layout, 50 µl Assay Diluent RD1 (provided with the kit) were added to each well. 200 µl standard dilution or diluted sample (e.g. 1:20 in Calibrator Diluent RD6H) per well were then added. The tip was changed after every pipetting step.

The plate was covered with the adhesive strip (provided with the kit), and incubated at room temperature for two hour on a rocking platform.

Each well was aspirated and washed, repeating the process three times for a total of four washes. Washing was performed by filling each well with 400 µl Wash Buffer (provided with the kit), using a manifold dispenser, and subsequent aspiration. After the last wash, any remaining Wash Buffer was removed by aspirating. The plate was then inverted and blotted against clean paper towels.

The aspirated samples and wash solutions were treated with laboratory disinfectant, and 200 µl of Conjugate (provided with the kit) were added to each well. The plate was then covered with a new adhesive strip, and incubated at room temperature for two hours.

The aspiration and wash steps were repeated as described previously.

200 µl Substrate Solution (provided with the kit) were added to each well, followed by incubation for 20 minutes at room temperature, under protection from light. 50 µl Stop Solution (provided with the kit) were added to each well, followed by careful mixing.

The optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm.

EGF standard curve:

The standards provided with the ELISA kit produced a linear standard curve. Also very small concentrations showed detectable results.

EGF samples:

A total of four assays with the samples were performed. Each sample was measured 2 - 5 times, the number of determinations being dependent on the quality of the results (mean +/- SD) and the availability of sufficient amounts of serum.

When samples were diluted 1:20 in Calibrator Diluent RD6H, all samples were within the linear range of the ELISA.

TGF-alpha

Reagents, standard dilutions and samples were prepared following the manufacturer's instructions. Excess antibody-coated microtiter plate strips (provided with the kit) were removed from the frame to prepare an ELISA plate. After determining the required number of wells and the plate layout, 100 µl Assay Diluent RD1W (provided with the kit) were added to each well, followed by the addition of 50 µl standard dilution or sample per well. The tip was changed after every pipetting step.

The plate was covered with the adhesive strip provided with the kit, and incubated at room temperature for two hours, on a rocking platform.

Each well was aspirated and washed, repeating the process three times for a total of four washes. In the following wash step, each well was filled with 400 µl Wash Buffer (provided with the kit), using a manifold dispenser, followed by aspiration. After the last wash, any remaining Wash Buffer was removed by aspirating, and the plate was inverted and blotted against clean paper towels.

The aspirated samples and wash solutions were treated with laboratory disinfectant.

200 µl of TGF-alpha Cojugate (provided with the kit) were added to each well, and the plate was covered with a new adhesive strip, and incubated at room temperature for two hours.

The aspiration and wash steps were repeated, as describe above. Thereafter, 200 µl Substrate Solution (provided with the kit) were added to each well, and the plate was incubates at room temperature for 30 minutes, under protection from light.

50 µl Stop Solution (provided with the kit) were added to each well with careful mixing.

The optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm.

TGF-alpha standard curve:

The standards provided with the ELISA kit produced a linear standard curve. Also very small concentrations showed detectable results.

TGF-alpha samples:

A total of four assays with the samples was performed. Samples were measured in 2 – 4 independent assays.

Results

The correlation between the various markers was tested using Spearman's rank-order correlation coefficient test, and the results are shown in Figure 9. According to this test, correlation is ranked between –1 (for best negative correlation) and +1 (for best positive correlation). As shown in Figure 9, the serum levels of HER2, TGF-alpha, amphiregulinc and EGF showed very little correlation, which confirms that these genes act as independent markers.

Figure 10 shows the correlation of the markers tested with clinical covariates, including ECOG scores (BECOG = baseline ECOG score), prior chemotherapy (PRITCN), tumor burden, and duration of diagnosis (DIAGDUR, i.e. how long the subject had cancer prior to diagnosis). Lower ECOG scores (0 and 1) indicate that the disease is less severe and the patient is in relatively good condition. Higher ECOG scores (>1) indicate increasing severity from scores 2 to

4. As shown in Figure 10, there was no significant correlation between the serum levels of the tested markers the severity of the disease. On the other hand, amphireguling and EGF serum levels were significantly higher in subject who were subjected to more than 4 prior chemotherapy treatments.

Survival curves were plotted according to the Kaplan-Meier method. These curves were compared among subgroups of patients using the log-rank test, in order to define the cutoffs giving the best discrimination in defining the likelihood of progression-free survival (PFS) and overall survival (OS). The results for PFS and OS are shown in Figures 11 and 12, respectively. As shown in Figure 11, the cutoff point in terms of PFS is particularly clear for EGF levels (clear positive correlation).

The distribution of patients according to the cutoffs, using PFS, is shown in Figure 13. As shown in the Figure, the cutoffs determined works well for the inidividual markers RGF-alpha and EGF, and are particularly useful as a function of these to markers combined.

Survival curves were calculated by Kaplan–Meier survival analysis. The effect of HER2 levels on PFS and OS is illustrated by the Kaplan-Meier plots shown in Figure 14. The effect of TGF-alpha levels on PFS and OS is illustrated by the Kaplan-Meier plots shown in Figure 15. The effect of the EGF levels of PFS and OS is illustrated by the Kaplan-Meier plots shown in Figure 16.

EXAMPLE 2

SERUM BIOMARKER ANALYSIS IN PATIENTS WITH OVARIAN, PRIMARY PERITONEAL, OR FALLOPIAN TUBE CANCER TREATED WITH PERTUZUMAB AND CHEMOTHERAPY

Study Design

A Phase II, randomized, placebo-controlled, double-blind, multicenter clinical trial is performed in order to make a preliminary assessment of the efficacy of pertuzumab (rhuMAb 2C4) in combination with the chermotherapeutic agent, gemcitabine relative to gemcitabine in combination with placebo in subjects with platinum-resistant ovarian, primary peritoneal, or fallopian tube cancer, as measured by progression-free survival (PFS) for all subjects. Another objective of the trial is to evaluate the safety and tolerability of pertuzumab in combination with gemcitabine relative to gemcitabine in combination with placebo in subjects with platinum-resistant ovarian, peritoneal, or fallopian tube cancer.

Subjects who have experienced disease progression at or within 6 months of receiving a platinum-based chemotherapy regimen administered for advanced disease are eligible for this study. No more than one prior regimen for platinum-resistant disease is allowed.

Subjects are randomized in a 1:1 ratio to either treatment Arm 1 (gemcitabine + pertuzumab) or Arm 2 (gemcitabine + placebo). Gemcitabine is administered on Days 1 and 8 of a 21-day cycle. Gemcitabine is infused over 30 minutes (± 5 minutes) at a starting dose of 800 mg/m^2. Blinded study drug (gemcitabine or placebo) is administered on Day 1 of the 21-day cycle, 30 minutes following gemcitabine administration. Pertuzumab is administered at an initial loading dose of 840 mg (Cycle 1), followed by 420 mg for Cycle 2 and beyond. The matched placebo is administered at a volume equivalent to the amount of suspension fluid required to prepare the pertuzumab dose.

Subjects without progressive disease are allowed to receive treatment with gemcitabine plus blinded study drug for up to 17 cycles in this study. Response is assessed every 6 weeks for the first eight cycles and about every 3 months thereafter end of Cycles 2, 4, 6, 8, 12, and 17). Measurable disease is assessed using the Response Evaluation Criteria for Solid Tumors (RECIST) by clinical evaluation and computed tomography scan or equivalent. Response for subjects with evaluable disease is assessed according to changes to CA-125 and clinical radiologic evidence of disease.

Patients who provide additional consent have the option to provide serum and plasma samples for exploratory biologic marker studies. These studies include assessment of potential mutations in the HER receptor gene family, immunohistochemistry for HER family proteins and downstream proteins associated with HER signaling, dimerization assays or proximity assays ro assess HER2 activation, and determination of the expression levels of specific genes that are identified to be associated with HER2 signaling, or may serve as markers or predictors of response. The studies include gene expression analysis and proteomics techniques.

Primary outcome measure:

Progression-free survival, as determined by investigator assessment using RECIST or by CA-125 changes (subjects with non-measurable disease only).

Secondary outcome measures:

Objective response rate (partial response or complete response), duration of response, survival time, and freedom from progression at 4 months.

Primary endpoint:

The primary efficacy endpoint is progression-free survival, defined as the time from randomization to documented disease progression or death from any cause on study, whichever occurs earlier. Disease progression is assessed by the investigator according to RECIST or

changes in CA-125 for subjects with measurable and non-measurable disease, respectively. Death on study is defined as death from any cause within 30 days of the last dose of study medication.

Data for subjects without disease progression or death is censored at the time of the last tumor or CA-125 assessment (or, if no tumor or CA-125 assessment are performed after the baseline visit, at the time of randomization plus 1 day).

Kaplan-Meier methods are used to estimate the median progression-free survival for each treatment arm. Cox proportional hazard models, using two models (with and without the randomization stratification factors [Eastern Cooperative Oncology Group (ECOG) status disease measurability and number of prior regimens for platinum-resistant disease], are used to estimate the hazard ratio (i.e., the magnitude of treatment effect at 95% confidence interval). The stratified model produces the primary confidence interval. The log-rank test, stratified by the randomization stratification factors (ECOG status, disease measurability, and number of prior regomens for platinum-resistant disease), is used to perform exploratory hypothesis testing for assessing the difference between treatment arms. The non-stratified long-rank test is also provided. Separate analyses of progression-free survival are also presented for subjects with measurable disease and for subjects with non-measurable disease; because the number of subjects in each grou pmay be small, the exploratory log-rank test may not be performed for both groups. Separate analyses for progression-free survival are also be conducted for subjects who do not have any prior regimen for platinum-resistant disease and for subjects who have one prior regimen for platinum-resistant disease; exploratory log-rank tests are performed for both groups.

Secondary endpoints:

Objective response

Objective response is defined as a complete or partial response determined on two consecutive occasions ≥4 weeks apart. Subjects without a post-baseline tumor or CA-125 assessment are considered non-responders. An estimate of the objective response rate and 95% confidence intervals (Blyth-Still-Casella) is calculated for each treatment arm. Confidence intervals for the difference in tumor response rate are calculated (Santer and Snell, *J. Am. Stat.Assoc.* 75:386-94 (1980); Berger and Boos, *J. Am. Stat. Assoc.* 89:4087-91 (1990)). Fisher's exact test is used to perform exploratory hypothesis testing for exploring the difference between treatment arms.

Duration of objective response

For subjects with an objective response, duration of the objection response is defined as the time from the initial response to disease progression or death from any cause on study.

Methods for handling censoring and for analysis are the same as described for progression-free survival.

Freedom from progression at 4 months

The proportion of subjects free from progression at 4 months for each treatment arm is estimated from the Kaplan-Meier curve for progression-free survival. An estimate of the progression-free rate and 95% confidence intervals (Greemwood, *Rep. Pub. Health. Med. Subjects* 33:1-26 (1926)) is calculated for each treatment arm. A two-sided Z-test is used to perform exploratory hypothesis testing for assessing the difference between treatment arms.

Duration of Survival

Duration of survival is defined as the time from randomization until death from any cause. Alld eaths are included, whether they occur on study or following treatment discontinuation. For subjects who have not died, duration of surival is censored at the date of last contact. Analysis methods are the same as those described for progression-free-survival.

The study is ongoing, but it is expected that, based on the gene expression analysis of serum or plasma samples, patients producing an elevated level of epidermal growth factor (EGF) and/or transforming growth factor alpha (TGF-alpha) will show an extended survival (especially progression-free survival) in response to pertuzumab and gemcitabine treatment.

WHAT IS CLAIMED IS:

1. A method for extending survival of a cancer patient comprising administering a HER dimerization inhibitor to the patient in an amount which extends survival of the patient, wherein the patient is determined to produce an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha), and the cancer is selected from the group consisting of ovarian cancer, peritoneal cancer and fallopian rube cancer.

2. The method of claim 1 wherein the patient is determined to produce an elevated level of EGF.

3. The method of claim 2 wherein the patient is found to have an elevated level of EGF in serum of the patient.

4. The method of claim 1 wherein the patient is determined to produce an elevated level of TGF-alpha.

5. The method of claim 4 wherein the patient is found to have an elevated level of TGF-alpha in serum of the patient.

6. The method of claim 1 wherein the HER dimerization inhibitor is a HER2 dimerization inhibitor.

7. The method of claim 1 wherein the HER dimerization inhibitor inhibits HER heterodimerization.

8. The method of claim 1 wherein the HER dimerization inhibitor is a HER antibody.

9. The method of claim 8 wherein the antibody binds to a HER receptor selected from the group consisting of EGFR, HER2, and HER3.

10. The method of claim 9 wherein the antibody binds to HER2.

11. The method of claim 10 wherein the HER2 antibody binds to Domain II of HER2 extracellular domain.

12. The method of claim 11 wherein the antibody binds to a junction between domains I, II and III of HER2 extracellular domain.

13. The method of claim 12 wherein the HER antibody comprises the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, respectively.

14. The method of claim 13 wherein the HER dimerization inhibitor is pertuzumab.

15. The method of claim 8 wherein the HER antibody is a naked antibody.

16. The method of claim 8 wherein the HER antibody is an intact antibody.

17. The method of claim 8 wherein the HER antibody is an antibody fragment comprising an antigen binding region.

18. The method of any one of claims 1-17 wherein the cancer is advanced, refractory or recurrent ovarian cancer.

19. The method of any one of claims 1-17 wherein the cancer is platinum resistant ovarian cancer.

20. The method of any one of claims 1-17 wherein the cancer is primary peritoneal or fallopian tube cancer.

21. The method of any one of claims 1-17 wherein the HER dimerization inhibitor is administered as a single anti-tumor agent.

22. The method of any one of claims 1-17 comprising administering a second therapeutic agent to the patient.

23. The method claim 22 wherein the second therapeutic agent is selected from the group consisting of chemotherapeutic agent, HER antibody, antibody directed against a tumor associated antigen, anti-hormonal compound, cardioprotectant, cytokine, EGFR-targeted drug, anti-angiogenic agent, tyrosine kinase inhibitor, COX inhibitor, non-steroidal anti-inflammatory drug, farnesyl transferase inhibitor, antibody that binds oncofetal protein CA 125, HER2 vaccine, HER targeting therapy, Raf or ras inhibitor, liposomal doxorubicin, topotecan, taxane, dual tyrosine kinase inhibitor, TLK286, EMD-7200, a medicament that treats nausea, a medicament that prevents or treats skin rash or standard acne therapy, a medicament that treats or prevents diarrhea, a body temperature-reducing medicament, and a hematopoietic growth factor.

24. The method of claim 23 wherein the second therapeutic agent is a chemotherapeutic agent.

25. The method of claim 24 wherein the chemotherapeutic agent is an antimetabolite chemotherapeutic agent.

26. The method of claim 25 wherein the antimetabolite chemotherapeutic agent is gemcitabine.

27. The method of claim 22 wherein the second therapeutic agent is trastuzumab, erlotinib, or bevacizumab.

28. The method of claim 1 wherein progression free survival (PFS) is extended.

29. The method of claim 1 wherein overall survival (OS) is extended.

30. A method for extending survival of a patient with ovarian, peritoneal, or fallopian tube cancer comprising administering pertuzumab to the patient in an amount which extends survival of the patent, wherein the patient is determined to produce an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha).

31. The method of claim 30 wherein patient has ovarian cancer.

32. The method of claim 30 or claim 31 wherein the patient has advanced, refractory or recurrent ovarian cancer.

33. The method of any one of claims 30-32 further comprising administering a chemotherapeutic agent to the patient.

34. The method of claim 33 wherein the chemotherapeutic agent is an antimetabolite chemotherapeutic agent.

35. The method of claim 34 wherein the antimetabolite chemotherapeutic agent is gemcitabine.

36. A method for extending progression free survival (PFS) of a patient with ovarian, peritoneal, or fallopian tube cancer comprising administering pertuzumab to the patient in an amount which extends PFS in the patent, wherein the patient's serum is determined to have an elevated level of epidermal growth factor (EGF) therein.

37. A method for extending progression free survival (PFS) of a patient with ovarian, peritoneal, or fallopian tube cancer comprising administering pertuzumab to the patient in an amount which extends PFS in the patent, wherein the patient's serum is determined to have an elevated level of epidermal growth factor (EGF) and transforming growth factor alpha (TGF-alpha) therein.

38. The method of claim 26 or claim 37, wherein the cancer is ovarian cancer.

39. The method of claim 38 wherein the ovarian cancer is advanced, refractory or recurrent ovarian cancer.

40. A method of selecting a patient for treatment with a HER dimerization inhibitor, comprising treating the patient with the HER dimerization inhibitor if the patient is determined to produce an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha).

41. The method of claim 40 wherein the survival of the patient is extended relative to the survival of a patient who does not produce an elevated level of EGF or TGF-alpha and receives the same treatment.

42. The method of claim 41 wherein the survival is overall survival (OS).

43. The method of claim 41 wherein the survival is progression free survival (PFS).

44. The method of claim 41 wherein the HER dimerization inhibitor is a HER2 dimerization inhibitor.

45. The method of claim 41 wherein the HER dimerization inhibitor inhibits HER heterodimerization.

46. The method of claim 31 wherein the HER dimerization inhibitor is a HER antibody.

47. The method of claim 46 wherein the antibody binds to a HER receptor selected from the group consisting of EGFR, HER2, and HER3.

48. The method of claim 47 wherein the antibody binds to HER2.

49. The method of claim 48 wherein the HER2 antibody binds to Domain II of HER2 extracellular domain.

50. The method of claim 49 wherein the antibody binds to a junction between domains I, II and III of HER2 extracellular domain.

51. The method of claim 50 wherein the HER antibody comprises the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, respectively.

52. The method of claim 51 wherein the HER dimerization inhibitor is pertuzumab.

53. The method of claim 46 wherein the HER antibody is a naked antibody.

54. The method of claim 46 wherein the HER antibody is an intact antibody.

55. The method of claim 46 wherein the HER antibody is an antibody fragment comprising an antigen binding region.

56. The method of any one of claims 40-55, further comprising treating said patient with a chemotherapeutic agent.

57. The method of claim 56 wherein the chemotherapeutic agent is gemcitabine.

58. A kit comprising a HER dimerization inhibitor and a package insert or label indicating a beneficial use for the HER dimerization inhibitor if the patient to be treated produces an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha).

59. The method of claim 58 wherein the cancer is ovarian cancer, peritoneal or fallopian tube cancer.

60. The method of claim 38 wherein the beneficial use is extension of survival.

61. The method of claim 60 wherein the survival is progression-free survival.

62. The method of any one of claims 58-61 wherein the HER dimerization inhibitor is an antibody.

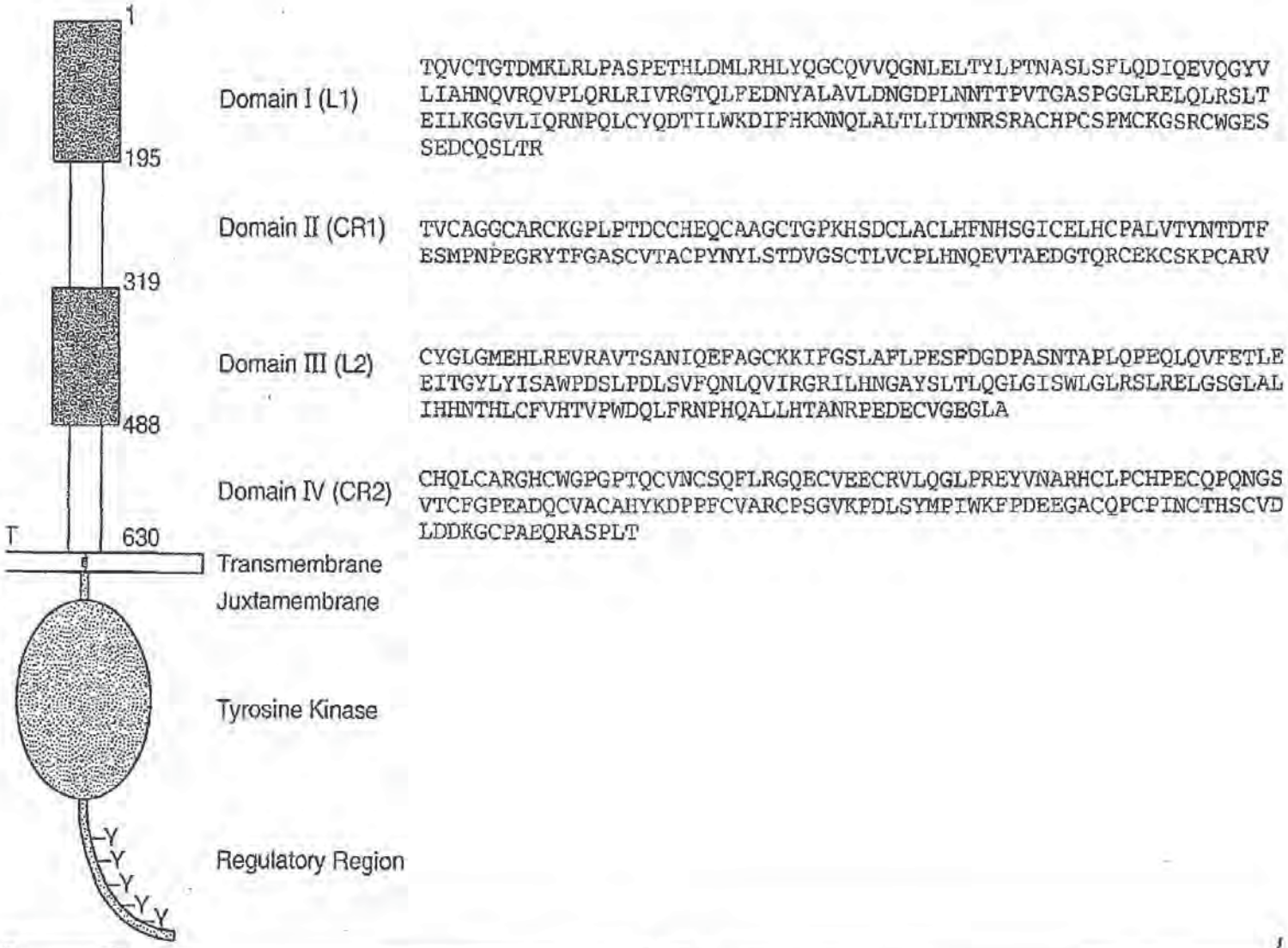
63. The method of claim 62 wherein the antibody is a HER2 antibody.

64. The method of claim 63 wherein the antibody is pertuzumab.

65. A method of promoting a HER dimerization inhibitor to treat patients producing an elevated level of epidermal growth factor (EGF) or transforming growth factor alpha (TGF-alpha).

66. The method of claim 65 wherein the promotion is in the form of a written material.

67. The method of claim 66 wherein the promotion is in the form of a package insert.



TQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQDIQEVQGYV
LIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLT
EILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGES
SEDCQSLTR

TVCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTF
ESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKCSKPCARV

CYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLE
EITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLAL
IHHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLA

CHQLCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHPECQPQNGS
VTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVD
LDDKGCPAEQRASPLT

FIG 1

VARIABLE LIGHT

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

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

                90         100
2C4     EDLAVYYC [QQYYIYPYT] FGGGTKLEIK (SEQ ID NO:1)
           * *                *   *
574     EDFATYYC [QQYYIYPYT] FGQGTKVEIK (SEQ ID NO:3)
                    *** *
hum κI  EDFATYYC [QQYNSLPWT] FGQGTKVEIK (SEQ ID NO:5)
```

FIG. 2A

VARIABLE HEAVY

```
                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 (SEQ ID NO:2)
        ***  **                          **
574     QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS (SEQ ID NO:4)
                           ********
hum III QMNSLRAEDTAVYYCAR [GRVGYSLYDY] WGQGTLVTVSS (SEQ ID NO:6)
```

FIG. 2B

Amino Acid Sequence for Pertuzumab Light Chain

```
1         10        20        30        40        50        60
|         |         |         |         |         |         |
DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPS

          70        80        90        100       110       120
          |         |         |         |         |         |
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPP

          130       140       150       160       170       180
          |         |         |         |         |         |
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT

          190       200       210
          |         |         |
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
```

FIG. 3A

Amino Acid Sequence for Pertuzumab Heavy Chain

```
1         10        20        30        40        50        60
|         |         |         |         |         |         |
EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIY

          70        80        90        100       110       120
          |         |         |         |         |         |
NQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSA

          130       140       150       160       170       180
          |         |         |         |         |         |
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG

          190       200       210       220       230       240
          |         |         |         |         |         |
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP

          250       260       270       280       290       300
          |         |         |         |         |         |*
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

          310       320       330       340       350       360
          |         |         |         |         |         |
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM

          370       380       390       400       410       420
          |         |         |         |         |         |
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ

          430       440       448
          |         |         |
QGNVFSCSVMHEALHNHYTQKSLSLSPG
```

FIG. 3B

FIG. 4

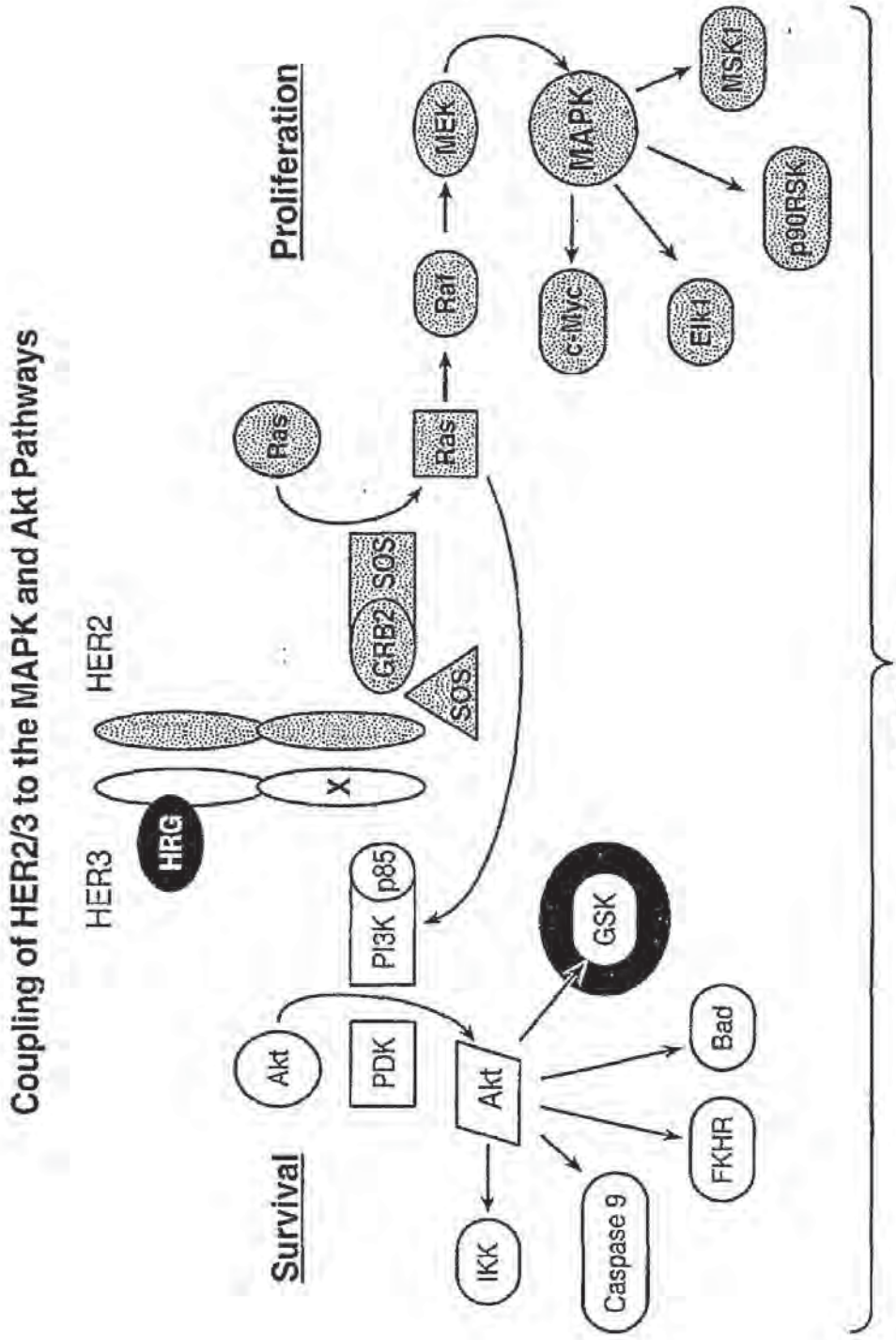
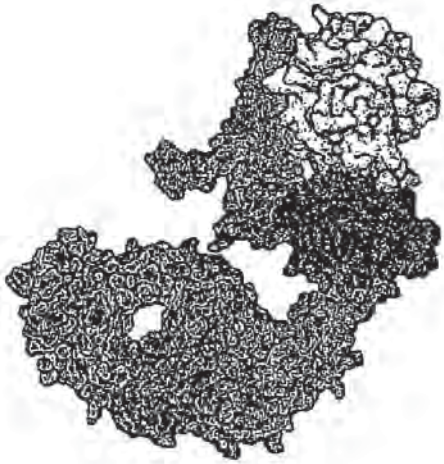

Coupling of HER2/3 to the MAPK and Akt Pathways
HER3
HER2
HRG
GRB2
SOS
SOS
Ras
Ras
Raf
MEK
MAPK
MSK1
p90RSK
c-Myc
Elk1
Proliferation
PI3K
p85
GSK
Akt
PDK
Akt
Bad
FKHR
IKK
Caspase 9
Survival

FIG. 5

Trastuzumab
Herceptin



- Binds in IV near JM.
- Protects against receptor shedding
- Moderately affects receptor down-modulation
- Slight effect on HER2's role as a coreceptor

Pertuzumab
Omnitarg

- Binds in II at dimerization interface
- Does not prevent receptor shedding
- Moderately affects receptor down-modulation
- Major effect on HER2's role as a coreceptor

FIG. 6

LIGHT CHAIN

```
1                               15                              30                              45
D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V N T A V A W Y Q Q K P G K A P K

46                              60                              75                              90
L L I Y S A S F L Y S G V P S R F S G S R S G T D F T L T I S S L Q P E D F A T Y Y C Q Q

91                              105                             120                             135
H Y T T P P T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L

136                             150                             165                             180
L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T

181                             195                             210     214
L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C
```

FIG. 7A

HEAVY CHAIN

```
1                               15                              30                              45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K D T Y I H W V R Q A P G K G L

46                              60                              75                              90
E W V A R I Y P T N G Y T R Y A D S V K G R F T I S A D T S K N T A Y L Q M N S L R A E D

91                              105                             120                             135
T A V Y Y C S R W G G D G F Y A M D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S

136                             150                             165                             180
K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S

181                             195                             210                             225
G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K

226                             240                             255                             270
T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S

271                             285                             300                             315
H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D

316                             330                             345                             360
W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E

361                             375                             390                             405
M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G

406                             420                             435                             449
S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G
```

FIG. 7B

```
1                               15                              30                              45
V H S D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q D V S I G V A W Y Q Q K P G K

46                              60                              75                              90
A P K L L I Y S A S Y R Y T G V P S R F S G S G S G T D F T L T I S S L Q P E D F A T Y Y

91                              105                             120                             135
C Q Q Y Y I Y P Y T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V

136                             150                             165                             180
V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S

181                             195                             210             217
T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C
```

FIG. 8A

```
1                               15                              30                              45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F T D Y T M D W V R Q A P G K G L

46                              60                              75                              90
E W V A D V N P N S G G S I Y N Q R F K G R F T L S V D R S K N T L Y L Q M N S L R A E D

91                              105                             120                             135
T A V Y Y C A R N L G P S F Y F D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S K

136                             150                             165                             180
S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G

181                             195                             210                             225
L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K T

226                             240                             255                             270
H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H

271                             285                             300                             315
E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W

316                             330                             345                             360
L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E M

361                             375                             390                             405
T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S

406                             420                             435                             449
F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G K
```

FIG. 8B

Figure 9

Table of correlation between markers (spearman correlation)

	HER2	TGF- α	AMPH IREG	EGF
HER2	1	-.2	.11	-.18
TGF-α		1	.2	.26
AMPHI			1	.07
EGF				1

Figure 10

Mean/Correlation of markers w/ clinical covariates

		HER2	TGF-α	AMPHI	EGF
BECOG	*n*	*96*	*96*	*93*	*95*
0	53	15.50	13.30	41.69	240.54
1	42	13.54	11.05	28.40	394.35
>1	1	12.53	13.39	11.15	125.45
PRITCN					
<=4	46	13.94	14.40	19.5	293.37
>4	50	15.22	10.40	51.06	320.43
TUMOR BURDEN	93	.04	.09	0	.02
DIAGDUR	96	-.09	.07	.04	.1

Figure 11

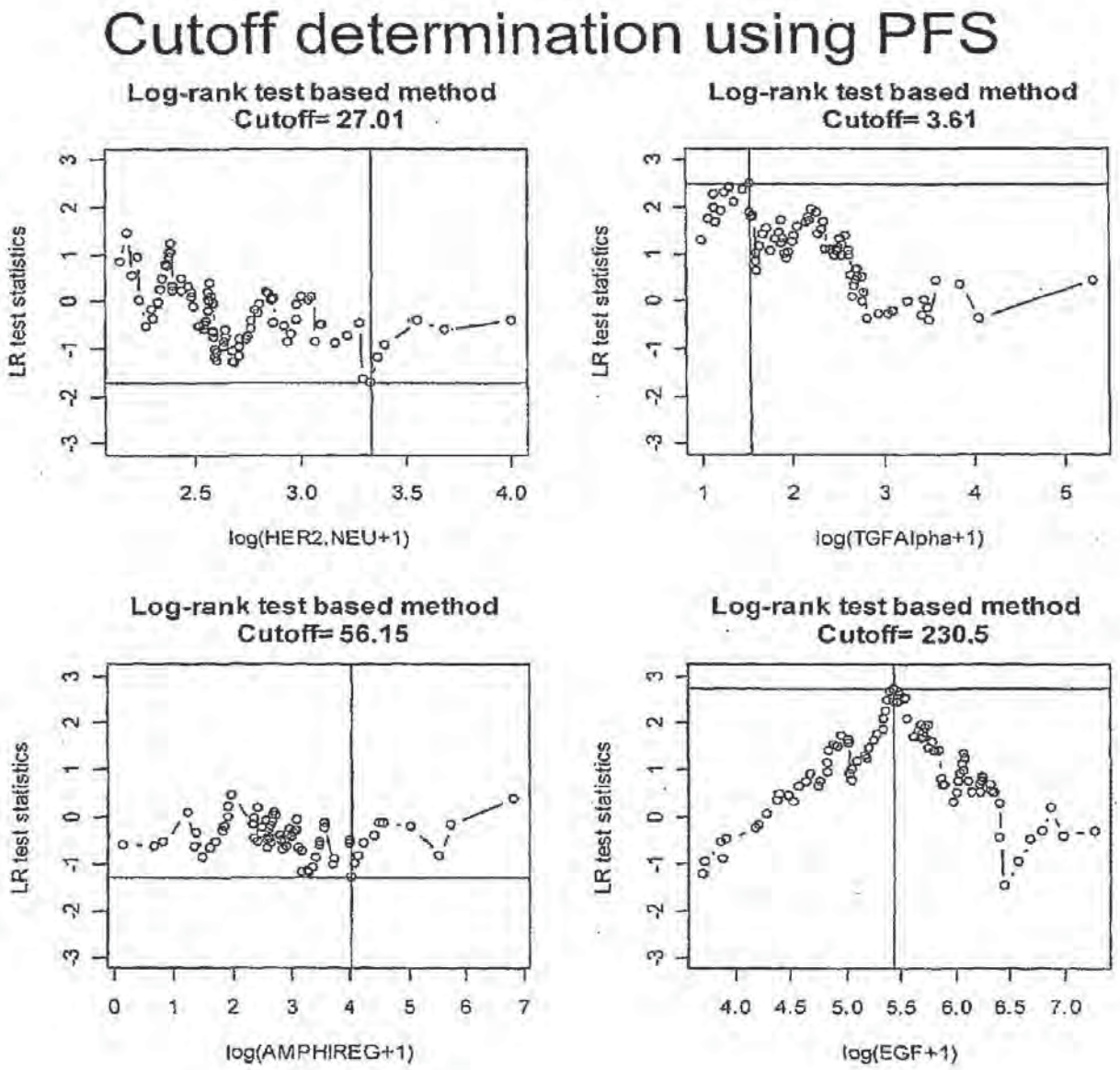
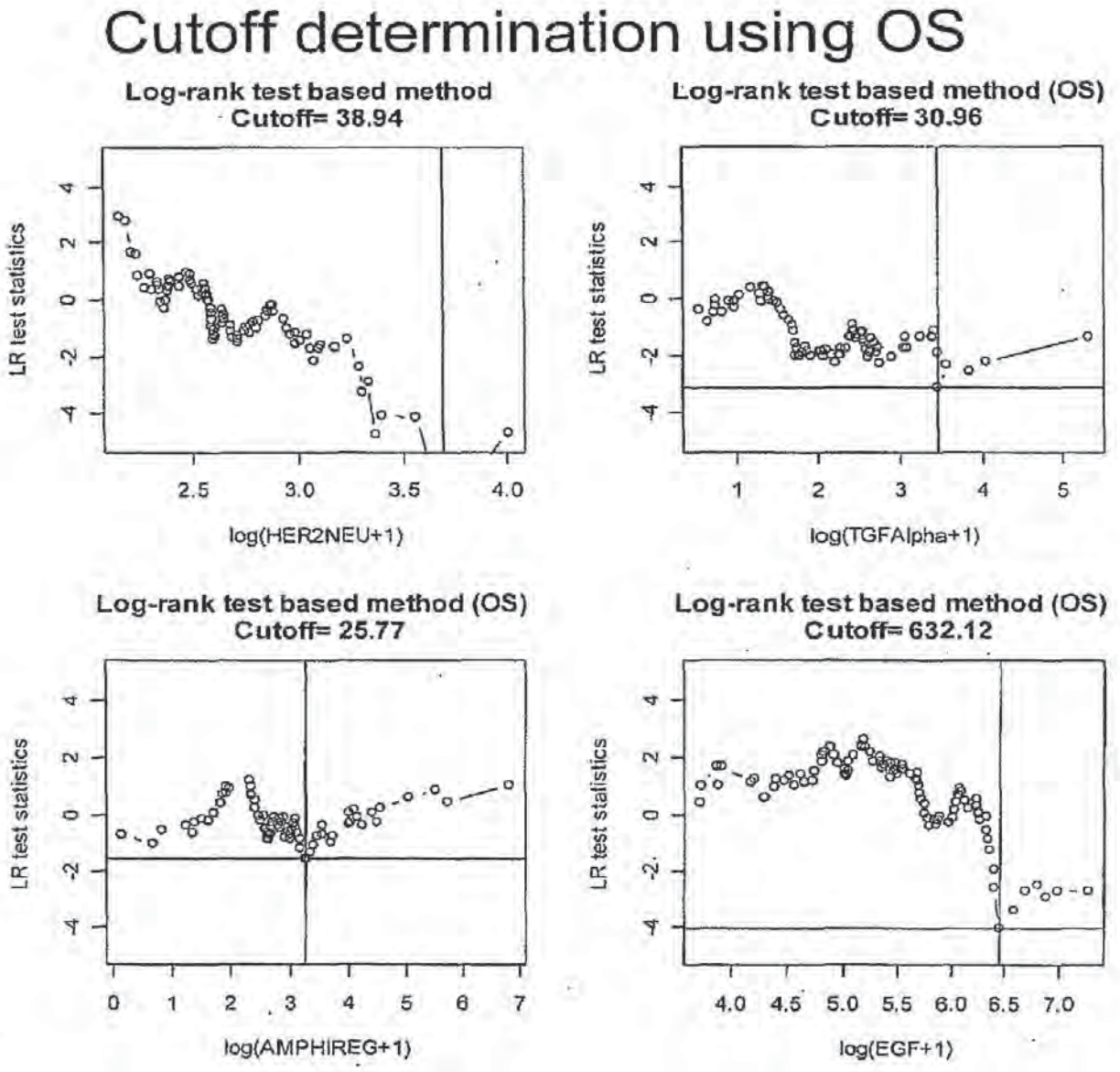

Cutoff determination using PFS
Log-rank test based method
Cutoff= 27.01
LR test statistics
log(HER2.NEU+1)
Log-rank test based method
Cutoff= 3.61
LR test statistics
log(TGFAlpha+1)
Log-rank test based method
Cutoff= 56.15
LR test statistics
log(AMPHIREG+1)
Log-rank test based method
Cutoff= 230.5
LR test statistics
log(EGF+1)

Figure 12


Cutoff determination using OS
Log-rank test based method
Cutoff= 38.94
LR test statistics
log(HER2NEU+1)
Log-rank test based method (OS)
Cutoff= 30.96
LR test statistics
log(TGFAlpha+1)
Log-rank test based method (OS)
Cutoff= 25.77
LR test statistics
log(AMPHIREG+1)
Log-rank test based method (OS)
Cutoff= 632.12
LR test statistics
log(EGF+1)

Distribution of patients according to cutoffs

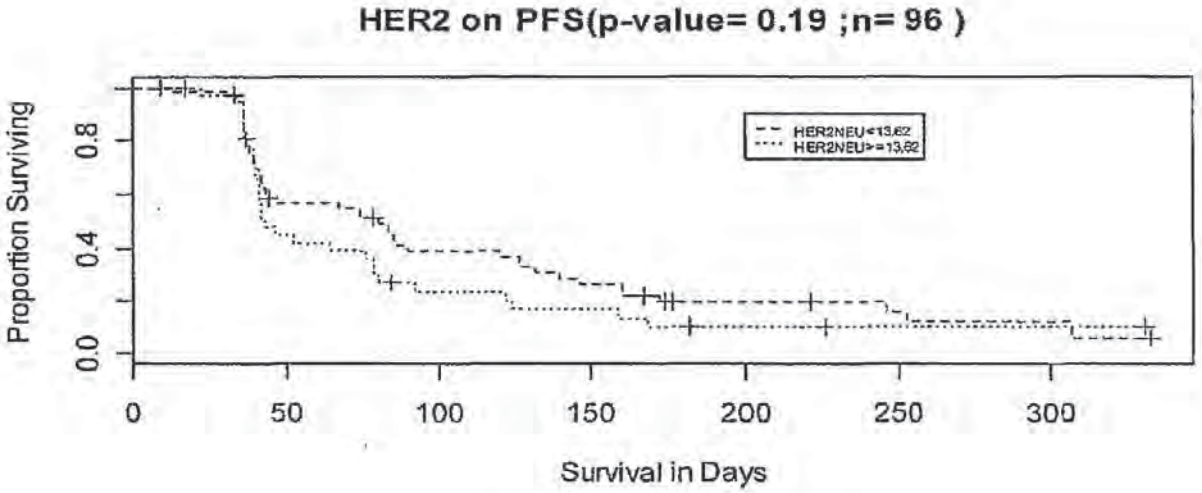
	perc.TRUE	perc.FALSE
HER2>=13.62	40	60
TGF>=8.06	47	53
AMPHI>=56.15	12	88
EGF>=230.5	54	46
TGF=HI, EGF=HI	29	71

(a function of two markers bothHI)

Figure 13

Figure 14

HER2



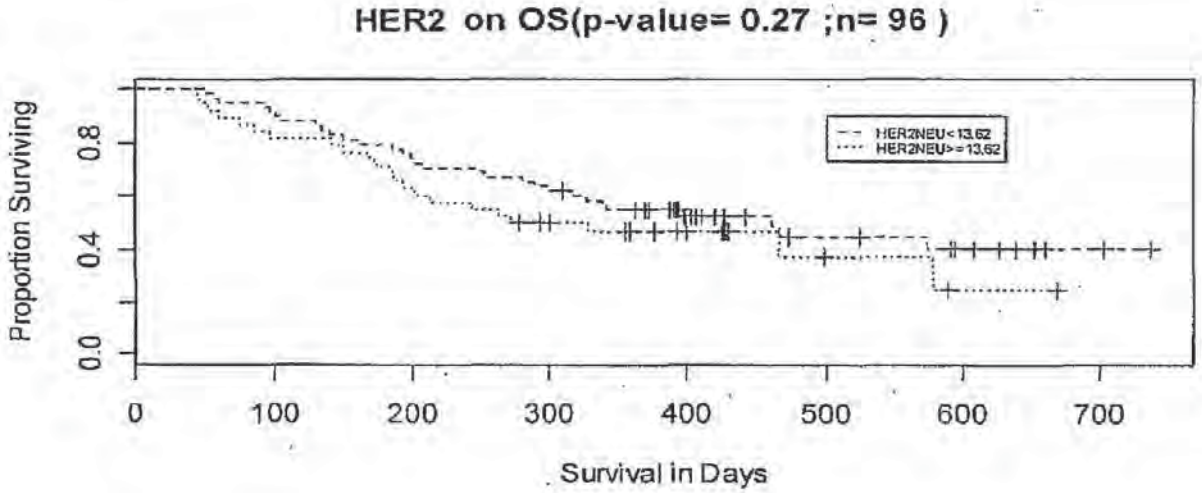


Figure 15

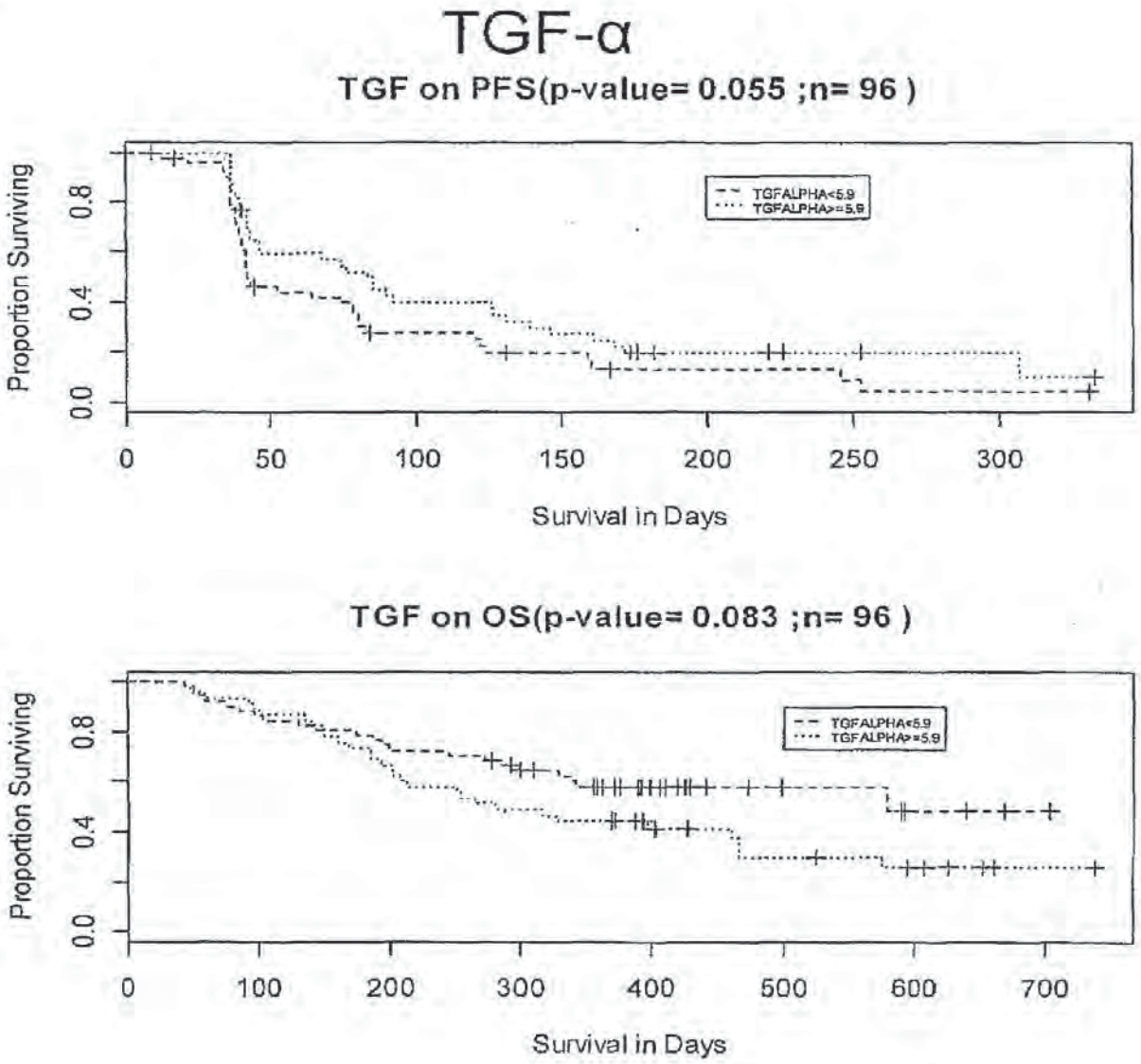
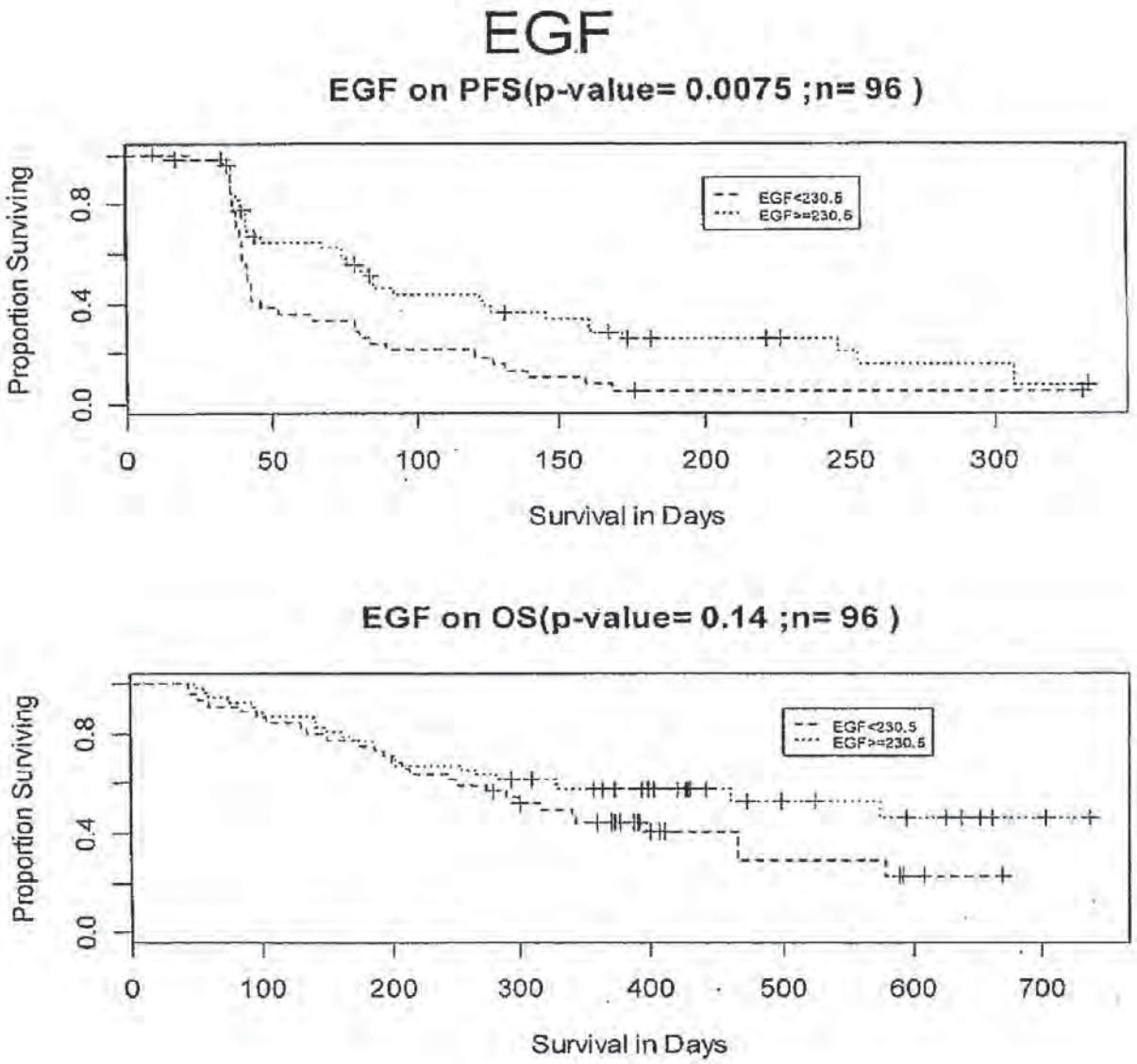

TGF-α
TGF on PFS(p-value= 0.055 ;n= 96)
Proportion Surviving
0.0
0.4
0.8
TGFALPHA<5.9
TGFALPHA>=5.9
0
50
100
150
200
250
300
Survival in Days
TGF on OS(p-value= 0.083 ;n= 96)
Proportion Surviving
0.0
0.4
0.8
TGFALPHA<5.9
TGFALPHA>=5.9
0
100
200
300
400
500
600
700
Survival in Days

Figure 16


EGF
EGF on PFS(p-value= 0.0075 ;n= 96)
Proportion Surviving
0.0
0.4
0.8
EGF<230.5
EGF>=230.5
0
50
100
150
200
250
300
Survival in Days
EGF on OS(p-value= 0.14 ;n= 96)
Proportion Surviving
0.0
0.4
0.8
EGF<230.5
EGF>=230.5
0
100
200
300
400
500
600
700
Survival in Days

Electronic Patent Application Fee Transmittal

Application Number:	10356824
Filing Date:	03-Feb-2003
Title of Invention:	Treatment with anti-ErbB2 antibodies
First Named Inventor/Applicant Name:	Virginia E. Paton
Filer:	Wendy M. Lee/Anna Kan
Attorney Docket Number:	P1256R1D1

Filed as Large Entity

Utility Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	2836738
Application Number:	10356824
International Application Number:	
Confirmation Number:	4326
Title of Invention:	Treatment with anti-ErbB2 antibodies
First Named Inventor/Applicant Name:	Virginia E. Paton
Customer Number:	9157
Filer:	Wendy M. Lee/Anna Kan
Filer Authorized By:	Wendy M. Lee
Attorney Docket Number:	P1256R1D1
Receipt Date:	08-FEB-2008
Filing Date:	03-FEB-2003
Time Stamp:	18:05:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$180
RAM confirmation Number	2856
Deposit Account	070630
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes) /Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Filed	P1256R1D1IDS.pdf	313557 e6eb67ba31961799cbbf4c5bc9e13bcc1c8f7eb6	no	6
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
2	Foreign Reference	WO2000069460.pdf	2095062 0caa521a09ac64ef1cc3a3deb001a1d861a81db5	no	39
Warnings:					
Information:					
3	Foreign Reference	WO2007145862.pdf	5764377 10612ac23e4d63d14cd7b654006d17cfbf0fbc7d	no	100
Warnings:					
Information:					
4	NPL Documents	brodowicz.pdf	438005 5d37eb550a0a4bc9000f19a1d8d0e7eba5c92cb6	no	5
Warnings:					
Information:					
5	NPL Documents	brufsky.pdf	81198 d0220ee6801c0986474c1a020839fbbbd3c9ac52	no	1
Warnings:					
Information:					
6	NPL Documents	bunn.pdf	1012775 942fcca8fc029cbc1f6718934831 6ebc06fbc65	no	12
Warnings:					
Information:					
7	NPL Documents	chrisposter2.pdf	190762 db1d9c76a4b658960cf3035c4431650860cec498	no	13
Warnings:					
Information:					
8	NPL Documents	chrisabstract.pdf	49326 67a078b6719a1b4269a5ee2f495c9c2e6a81ca66	no	1
Warnings:					

Information:					
9	NPL Documents	equivocal.pdf	49569 fb41b5e5ab5da3c1fa571aca1375630e756c2865	no	1
Warnings:					
Information:					
10	NPL Documents	hirsch.pdf	112956 9335b1644e21ed633564afdf47a51307b2098cc1	no	2
Warnings:					
Information:					
11	NPL Documents	hirsch2002.pdf	402956 354334244c751458e6358fc202edcc33637435ad	no	6
Warnings:					
Information:					
12	NPL Documents	noguerasabstract.pdf	134117 fcfbbea4040ccbf58c2d40109eb25c7e125dde23	no	1
Warnings:					
Information:					
13	NPL Documents	oshaughnessy2004.pdf	1080801 149f7fd4e333d5d46a569d23ae478e785e521556	no	6
Warnings:					
Information:					
14	NPL Documents	oshaughnessy.pdf	95978 0dddff05e5c8c870241220249b7a7d169f9e1c00d	no	2
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Information:					
15	NPL Documents	peacock.pdf	62416 d101c0de92cddd50ab61080c331c598482e08794	no	1
Warnings:					
Information:					
16	NPL Documents	pegram.pdf	157379 3d66c21adfc799847cf3a87cda0c7e16040fda30	no	2
Warnings:					
Information:					
17	NPL Documents	robert.pdf	56303 d9054314a5d66be3e414a5d9b1b6483b8392924a	no	1
Warnings:					

Information:					
18	NPL Documents	vosko.pdf	295143 460a569492e72a4b6f2443ad9439d611be662c8	no	13
Warnings:					
Information:					
19	NPL Documents	yardley.pdf	52263 d227cd8a21840e8e9ccc48b9dd6c98d983e568b9	no	1
Warnings:					
Information:					
20	Fee Worksheet (PTO-06)	fee-info.pdf	8157 d7e5088bf70bb2558e2717f949217d655a3d3864	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	12453100		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (07-06)
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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
10/356,824	02/03/2003	

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY ☐ RATE ($)	SMALL ENTITY ☐ FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT 02/08/2008	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 17	Minus	** 33	= 0	X $ =		OR	X $50=	0
Independent (37 CFR 1.16(h))	* 3	Minus	***5	= 0	X $ =		OR	X $210=	0
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/PAUL M. STANBACK/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/356,824	02/03/2003	Virginia E. Paton	P1256R1D1	4326

9157 7590 03/12/2008

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

EXAMINER	
HOLLERAN, ANNE L	

ART UNIT	PAPER NUMBER
1643	

MAIL DATE	DELIVERY MODE
03/12/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Interview Summary	Application No.	Applicant(s)	
	10/356,824	PATON ET AL.	
	Examiner	Art Unit	
	Anne L. Holleran	1643	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Anne L. Holleran*. (3)_____.

(2) *Wendy Lee*. (4)_____.

Date of Interview: *28 January 2008*.

Type: a)☐ Telephonic b)☐ Video Conference
c)☒ Personal [copy given to: 1)☐ applicant 2)☒ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☐ No.
If Yes, brief description: _____.

Claim(s) discussed: _____.

Identification of prior art discussed: _____.

Agreement with respect to the claims f)☐ was reached. g)☒ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: _____

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

Discussed proposed amendments and arguments, which appear to overcome rejections of record under 112, first, 112, 2nd and 102(b). Applicants propose providing evidence to overcome 103(a) rejections.

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

Anne L Holleran
Examiner's signature, if required

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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/356,824	02/03/2003	Virginia E. Paton	P1256R1D1	4326

9157 7590 06/26/2008

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

EXAMINER	
HOLLERAN, ANNE L	
ART UNIT	PAPER NUMBER
1643	
MAIL DATE	DELIVERY MODE
06/26/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/356,824	PATON ET AL.	
	Examiner	Art Unit	
	ANNE L. HOLLERAN	1643	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 February 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *34-39,42-44 and 47-54* is/are pending in the application.

4a) Of the above claim(s) *50 and 51* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *34-39,42-44,47-49 and 52-54* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *02/08*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The amendment filed February 8, 2008 is acknowledged. Claims 20, 25-29, 32, 40-41, 45, and 46 were canceled. Claims 50-54 were added.

Claims 34-39, 42-44, 47-54 are pending.

New claims 50 and 51, drawn to inventions comprising the use of third therapeutic agents, which are not carboplatin (another ErbB2 antibody, claim 50; a vascular endothelial growth factor antibody, claim 51) are **withdrawn from consideration** in view of the election of species requirement for the third therapeutic agent set forth in the Office action mailed out 2/16/2006.

With respect to claims 52 and 53, which recite a third therapeutic agent is a growth inhibitory agent or a DNA alkylating agent, these claims are considered generic to the elected species of third agent, carboplatin, because carboplatin is a growth inhibitory agent and a DNA alkylating agent. Claims 52 and 53 are examined to the extent that they read on methods using a third agent that is carboplatin.

Claims 34-39, 42-44, 47-49 and 52-54 are examined on the merits.

Claim Rejections Withdrawn:

Claim Rejections - 35 USC § 112

The rejection of claim 43 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as

the invention is withdrawn in view of the amendment to claim 38 providing antecedent basis for "the taxoid" in claim dependent claim 43.

The rejection of claims 20, 25, 28, 29, 32, and 46 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is withdrawn in view of the amendment canceling claims 20, 25, 28, 29, 32 and 46.

The rejection of claims 38-40, and 43-49 under 35 U.S.C. 112, first paragraph, for lack of enablement of the full scope of the claimed inventions, is withdrawn in view of the amendment to the claims.

Claim Rejections - 35 USC § 102

The rejection of claims 20, 25-28, 32, 34-37, 48 and 49 under 35 U.S.C. 102(b) as being anticipated by Nabholtz-I (Nabholtz, J.M., et al, Breast Cancer Research and Treatment, 64(1): page 82, #327, 2000) or Nabholtz-II (Nabholtz, J. M., et al., European Journal of Cancer, 37(suppl. 6): S190, #695, 2001) is withdrawn in view of the amendment canceling claims 20, 25-28 and 32; and withdrawn for claims 34-37, 48 and 49 in view of the amendment to the claims so that now the claims have priority to parent application 60/069,346 (filed 12/12/1997).

Claim Rejections Maintained and New Grounds of Rejection:

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 52 and 53 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 52 and 53 are indefinite because claim 52 lacks antecedent basis for the phrase "the third therapeutic agent".

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 20, 25-28, 32, 34-49 is rejected under 35 U.S.C. 103(a) as being unpatentable over Baselga-1996 (Baselga, et al., Journal of Clinical Oncology, 14(3): 737-744, 1996, March; cited in the IDS) in view of Perez (Perez et al., Seminars in Oncology, 23(5, suppl 11): 41-45, 1996, October) and further in view of Baselga-1994a (Baselga, J., et al, Proceedings of the American Association for Cancer Research, 35: 380, Abstract #2262, 1994). This is a **new grounds** of rejection because Baselega-1994 has been replaced with Baselga-1994a (cited in the parent application).

Baselga-1996 teaches methods of treating breast cancer comprising the administration of trastuzumab, a recombinant humanized anti-Her2 antibody that binds to the 4D5 epitope. Baselga-1996 fails to teach a method comprising the further administration of a chemotherapeutic agent such as a taxoid and growth inhibitory agents. Baselga-1996 also fails to teach a method comprising the administration of trastuzumab in combination with a taxoid and further in combination with carboplatin.

However Perez teaches that the combination of paclitaxel and carboplatin may be used in the treatment of patients with advanced breast cancer, and that both agents have significant single-agent activity in the treatment of breast cancer (see page 43, 2nd column).

Baselga-1994 teaches a method of treating nude mice bearing human breast tumor xenografts overexpressing ErbB2 (HER2) comprising the administration of murine 4D5 (binds to the same epitope as rhuMAb 4D5) in combination with paclitaxel. Baselga-1994 teaches in general that chemotherapy synergizes with anti-growth factor receptor antibodies in the treatment of human tumor xenografts in nude mice. Baselga-1994 teaches that the combination of paclitaxel and the 4D5 antibody was synergistic in the effect of the combination on tumor growth inhibition.

In summary, the prior art teaches that rhuMAb 4D5 increases time to disease progression for metastatic breast cancer patients because Baselga teaches that treatment with the antibody produced a complete response and partial responses, as well as stable disease (see Table 4, and also page 742, 2nd column). Further, the prior art teaches that a combination of paclitaxel and carboplatin are used in the treatment of breast cancer. Lastly, the prior art provides evidence, in the form of mouse xenograft studies that the combination of 4D5 and paclitaxel is synergistic with respect to growth inhibition of a human breast tumor that overexpresses ErbB2. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have treated breast cancer patients overexpressing ErbB2 with a combination of an antibody that binds the 4D5 epitope, such as rhuMAb 4D5, and a taxoid, such as paclitaxel because both agents where taught in the prior art to be useful in treating breast cancer, and because the prior art suggested their combination, and because the prior art demonstrated that in

nude mouse xenograft studies the combination of the two agents was more efficacious in inhibiting tumor cell growth than either agent used alone and more efficacious than sum of the effects of either agent used alone. One would have had a reasonable expectation of success in making a method that was effective to extend TTP because both agents were taught in prior art to be effective to produce at least stable disease in the treatment of breast cancer. The methods of claims 20, 25-28, 32, 34-49 can be viewed as a methods drawn to administering a combination of ingredients known in the art to be useful for the same purpose, i.e. an In re Kerkhoven analysis (In re Kerkhoven, 626, F.2s 846, 850, 205 USPQ 1069, 1072 (CCPA 1980)). The court held that it is obvious to combine two compositions, in order to form a third composition, when each of the two compositions is taught by the prior art to be useful for the same purpose. The idea of combining them flows logically from their having been individually taught in the prior art (MPEP 2144.06).

Claims 38 and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Baselga-1996 (Baselga, et al., Journal of Clinical Oncology, 14(3): 737-744, 1996, March; cited in the IDS) in view of Perez (Perez et al., Seminars in Oncology, 23(5, suppl 11): 41-45, 1996, October) in view of Baselga-1994a (Baselga, J., et al, Proceedings of the American Association for Cancer Research, 35: 380, Abstract #2262, 1994; cited in the IDS), and further in view of van Oosterom (van Oosterom, et al. Anti-Cancer Drugs, 6(3): 356-368, 1995, June, Abstract only; cited in the IDS). This is a **new grounds** of rejection because Baselga-1994a replaces Baselga-1994.

Claims 38 and 43 include within their scope methods that comprise the administration of docetaxel.

The combination of Baselga-1996, Perez and Baselga-1994a is set forth above, and fails to explicitly teach methods comprising the administration of docetaxel.

However, van Oosterom teaches that docetaxel is a taxoid that has activity in the treatment of breast cancer. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Baselga-1996 to include the addition of docetaxel and further to also include the addition of a third agent, carboplatin. One would have been motivated to have combined trastuzumab with docetaxel and a third agent such as carboplatin because van Oosterom teaches that docetaxel is useful in the treatment of breast cancer, and because Perez teaches that carboplatin is useful in the treatment of breast cancer. The methods of claims 20, 29, 38 and 43 can be viewed as a methods drawn to administering a combination of ingredients known in the art to be useful for the same purpose, i.e. an In re Kerkhoven analysis (In re Kerkhoven, 626, F.2s 846, 850, 205 USPQ 1069, 1072 (CCPA 1980)). The court held that it is obvious to combine two compositions, in order to form a third composition, when each of the two compositions is taught by the prior art to be useful for the same purpose. The idea of combining them flows logically from their having been individually taught in the prior art (MPEP 2144.06).

Response to Arguments:

Applicants' arguments have been carefully considered, but fail to persuade.

Applicants make the following points:

1) that Baselga-1994 teaches away from the present claimed methods. This moot because Baselga-1994a replaces Baselga-1994;

2) that is was not reasonably predictable from the cited references, because the claims are drawn to treatment of a human patent;

3) that a cancer that over expresses ErbB2 receptor is a clinically aggressive cancer that predicts a worse prognosis, leading to unpredictability;

4) that applicants provide evidence that negates any inference that a skilled clinician might have obtained from the preclinical data;

5) that applicants submit a copy of a 1.132 declaration submitted in the parent on August 23, 2000 with exhibits A-E;

6) that in the declaration cited above Dr. Hellmann explains a further "unexpected advantage of the present claimed invention;

7) that applicants submit evidence of commercial success supports the patentability of the presently claimed invention;

In the traversal of the rejection, applicants address the issue of the xenograft study referred to on page 6 of Exhibit C to Hellmann's first 131 declaration dated 8/23/00 (mailroom date of 8/28/2000), by stating that the "different model system at UCLA" was referred to in Pegram et al. Oncogene 18: 2241-2251, 1999 (of record). Applicants state that in Pegram, in Figure 5C animals treated with rhuMAb HER2 alone actually had smaller tumor volumes at day 20 than those treated with a combination of a taxane compound (TAX) plus rhuMAb HER2. The examiner does not agree with this interpretation of the results of the data of Figure 5C. In the examiner's opinion the data for the two treatment conditions overlap to such an extent that it

cannot be determined if there is a difference between the two treatment groups. Furthermore, applicants state in their arguments that at paragraph 2, column 2 of page 2245 of Pegram, the authors noted the "the difference between rhuMAb HER2 alone and rhuMAb HER2 did not reach statistical significance."

The difference between the Pegram xenograft data and the Baselga 1994 xenograft data (Baselga abstract, of record, cited as prior art in the above rejection) is that while the Baselga 1994 xenograft data indicates that the combination of paclitaxel and the 4D5 anti-HER2 antibody are synergistic in the inhibition of tumor growth, the Pegram xenograft data indicates that the combination of paclitaxel and a 4D5 antibody is not different from the use of 4D5 alone (however, the combination of 4D5 and TAX (paclitaxel) is significantly more efficacious than TAX alone, see Figure 5C). While it is reasonable to state that the data from the two models are different, this difference between the two models does not appear to be contradictory or for one set of data to negate the other set. It is noted that in Figure 5C, the combination of 4D5 and TAX inhibits tumor growth to a greater degree than TAX alone. Furthermore, it is noted that for other chemotherapeutic agents tested in Figure 5C (DOX and CPA), the same pattern is observed, where the combination of chemotherapeutic agent and 4D5 antibody is more efficacious than chemotherapeutic agent alone. Thus, the Pegram data does not provide a "teaching away" from the claimed invention, because there is nothing in Pegram data that would indicate that the combination of paclitaxel (TAX) and a 4D5 anti-Her2 antibody would cause a decrease in the efficacy of either drug in the treatment of a human patient with a tumor characterized by overexpression of ErbB2 (Her2).

Applicants further state that they disagree with the conclusion about what would have been obvious to one of ordinary skill in the art prior to the instant invention.

1) Applicants submit that such a conclusion ignores the evidence concerning the unexpected advantage of avoiding the overall adverse side effects associated with other cancer drug combinations, such as an anti-ErbB2 antibody/anthracycline derivative combination (refers to pages 4-5 of Dr. Hellmann's 132 declaration dated 8/23/2000), which unexpected results, applicants state, are not disclosed in any of the cited references. In response to applicant's argument that the cited references fail to teach that there is an advantage in a decrease in side effects of the combination of a 4D5 anti-Her2 antibody and a taxoid, when compared to the side effects observed for the combination of a 4D5 anti-Her2 antibody and doxorubicin, the fact that applicant has recognized another advantage which would flow naturally from following the suggestion of the prior art cannot be the basis for patentability when the differences would otherwise be obvious. See *Ex parte Obiaya*, 227 USPQ 58, 60 (Bd. Pat. App. & Inter. 1985).

2) Applicants state that they have provided a declaration executed by a skilled clinician, Dr. Hellmann, attesting to the clinical data with respect to extending TTP in humans with the combination were unexpected to her. Applicants submit that this view held by a person actually practicing in the field should have been given more weight than an Examiner's interpretation of the data and what would have been expected or not. In response to this argument, it is not clear to the examiner what "data" applicants are referring to. Assuming it is the Pegram data that applicants are referring to, at the time the examiner made the rejection (previous Office action), no data was available to the examiner.

In giving deference to the statements of Dr. Hellmann in the declaration filed 8/28/2000, while Dr. Hellmann has made the statement that the extension of TTP for the combination was a surprising result, Dr. Hellmann has not supported this statement with evidence (the data provided supports that conclusion that there was synergism with the combination recited in the claims, not that the synergism was surprising). Therefore, the statement that the synergism was surprising appears to be a statement of an opinion. The MPEP states that in assessing the probative value of an expert opinion, the examiner must consider the nature of the matter sought to be established, the strength of any opposing evidence, the interest of the expert in the outcome of the case, and the presence or absence of factual support for the expert's opinion (MPEP 716.01(c)). In the instant case, applicants are arguing that the combination of a 4D5 antibody and paclitaxel exhibits synergism with respect to treatment of human patients with breast cancer, and that this synergism is a surprising result. In contrast the prior art provides that a 4D5 antibody was known to be effective in the treatment of breast cancer in humans, that paclitaxel was known to be effective in the treatment of breast cancer in humans, and that pre-clinical studies indicated that when the two treatments were combined for the treatment of mice bearing xenograft tumors that there was a synergistic result with respect to the slowing of growth of the tumors in vivo. In the instant case, the expert making the declaration of the surprising result is not a disinterested party, but is the named inventor of the claimed subject matter, and the declaration does not provide any facts to support the statement that the extension of TTP was a surprising result. As explained above in the discussion of the Pegram data, the rejection is maintained because the Pegram data definitely demonstrates a benefit of combining a 4D5 anti-Her2 antibody with paclitaxel over the use of paclitaxel alone, and because the Pegram data does

not provide a basis for believing that combining paclitaxel and a 4D5 anti-Her2 antibody would cause an overall decrease in TTP. Furthermore, the scope of the claimed inventions is broader than a method where the combination of treatment results in an extension of TTP over the use of either agent alone. The claimed invention only requires an extension of TTP with respect to no treatment at all.

3) Applicants state that the examiner ignores the additional secondary indicia of nonobviousness in terms of evidence of commercial success associated with the presently claimed invention (paragraph 8 of Dr. Hellmann's 132 declaration dated 8/23/2000). In response, paragraph 8 of Dr. Hellmann's 132 declaration dated 8/23/2000 (mailroom date of 8/28/2000) states that the "invention claimed in the above patent application has resulted in a dramatic change in the way oncologists treat human patients with ErbB2 overexpressing metastatic breast cancer." There is no mention of "evidence" of commercial success, simply the statement that the combination treatment covered by the present claims is used more often (by a factor of 2) than anthracycline use in the treatment of breast cancer. However, to address the issue of commercial success and whether the statement of paragraph 8 of Dr. Hellmann's 132 declaration is evidence of non-obviousness, it is noted that the MPEP states that applicant must show that the claimed features of the invention were responsible for the commercial success of an article if the evidence of nonobviousness is to be accorded substantial weight, and that merely showing that there was commercial success of an article which embodied the invention is not sufficient. The MPEP (716.03) states that an applicant who is asserting commercial success to support its contention of nonobviousness bears the burden of proof of establishing a nexus between the claimed invention and evidence of commercial success, and that the term "nexus"

designates a factually and legally sufficient connection between the evidence of commercial success and the claimed invention so that the evidence is of probative value in the determination of nonobviousness. In the instant case, before the filing date of the instant invention, anthracycline use was associated with cardiac toxicity (see for example Cottin, (Cottin, Y, et al. Br. Heart Journal, 73: 61-64, 1995) which teaches that while anthracylines are active chemotherapeutic agents, their efficacy is limited by cardiac toxicity, page 61, first column). Therefore, the higher rate of use of a therapy that does not include anthracyclines may have more to do with possible problems associated with anthracycline use than with the features of the claimed invention. Thus, simply presenting a comparison between a rate of use of the claimed invention and the rate of use of anthracycline is not probative of nonobviousness of the claimed invention.

Thus, for the reasons of record, and as stated herein, the rejection is maintained.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned

with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 34-39, 42-44, 47-49, and 52-54 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 4, and 8-29 of U.S. Patent No. 5,720,954 (cited in the IDS) in view of Baslega-1996 (Baselga, J. et al. Journal of Clinical Oncology, 14(3): 737-744, 1996, March; cited in IDS), in view of Perez (Perez et al., Seminars in Oncology, 23(5, suppl 11): 41-45, 1996, October; cited above) and further in view of Baselga-1994a (Baselga, J., et al, Proceedings of the American Association for Cancer Research, 35: 380, Abstract #2262, 1994).

Claims 1, 4 and 8-29 of US Patent No. 5,720,954 are drawn to methods of treating a patient having a carcinoma that overexpresses HER2 receptor comprising administering to said patient a cytotoxic factor and an antibody that binds to the HER2 receptor in amounts to eliminate the patient's tumor burden, where the antibody may be a 4D5 anti-HER2 antibody and where the cytotoxic factor may be a chemotherapeutic agent. The claims do not specifically recite a dosage. However, a dosage for an anti-4D5 Her2 antibody was known in the art as evidenced by the teachings of Baselga-1996. The claims do not specifically recite that the chemotherapeutic agent may be a taxoid, or that it be in combination with a further growth inhibitory agent. However, the use of taxoids and a further growth inhibiting agent such as carboplatin for the treatment of breast cancer is known in the prior art as evidenced by the teachings of either Perez, and the combination of a 4D5 anti-HER2 antibody and paclitaxel (a taxoid) exhibits synergism in reducing the growth of xenograft tumors in nude mice, as taught by

Baselga-1994a. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have used a taxoid such as paclitaxel in the treatment methods of claims 1, 4 and 8-29 of US Patent No. 5,720,954 to make the claimed methods.

Claims 34-39, 42-44, 47-49, and 52-54 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 14, 23, 27-33 and 36 of U.S. Patent No. 5,770,195 (cited in IDS) in view of Baslega-1996 (Baselga, J. et al. Journal of Clinical Oncology, 14(3): 737-744, 1996, March; cited in IDS), in view of Perez; cited previously) and further in view of Baselga-1994a (Baselga, J., et al, Proceedings of the American Association for Cancer Research, 35: 380, Abstract #2262, 1994).

Claims 14, 23, 27-33 and 36 of US Patent No. 5,770,195 are drawn to methods of of inhibiting the growth of tumor cells that overexpresses HER2 receptor or EGF comprising administering to a patient a cytotoxic factor and an antibody that binds to the HER2 receptor in amounts to eliminate the patient's tumor burden, where the antibody may be a 4D5 anti-HER2 antibody and where the cytotoxic factor may be a chemotherapeutic agent. The claims do not specifically recite a dosage. However, a dosage for an anti-4D5 Her2 antibody was known in the art as evidenced by the teachings of Baselga-1996. The claims do not specifically recite that the chemotherapeutic agent may be a taxoid, or that it be in combination with a further growth inhibitory agent. However, the use of taxoids and a further growth inhibiting agent such as carboplatin for the treatment of breast cancer is known in the prior art as evidenced by the teachings of either Perez, and the combination of a 4D5 anti-HER2 antibody and paclitaxel (a taxoid) exhibits synergism in reducing the growth of xenograft tumors in nude mice, as taught by

Baselga-1994a. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have used a taxoid such as paclitaxel in the treatment methods of claims 14, 23, 27-33 and 36 of US Patent No. 5,770,195 to make the claimed methods.

Claims 34-39, 42-44, 47-49, and 52-54 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-7 and 9-13 of U.S. Patent No. 6,387,371 in view of Baslega-1996 (Baselga, J. et al. Journal of Clinical Oncology, 14(3): 737-744, 1996, March; cited in IDS), in view of Perez; cited previously), and further in view of Baselga-1994a (Baselga, J., et al, Proceedings of the American Association for Cancer Research, 35: 380, Abstract #2262, 1994).

Claims 1-7 and 9-13 of US Patent No. 6,387,371 are drawn to methods of treating cancer that overexpresses HER2 receptor comprising administering to said patient a factor which suppresses cell growth in an amount effective to inhibit growth of the cancer in the patient, and an antibody that binds to an extracellular domain of the HER2 receptor. The claims do not specifically recite that the chemotherapeutic agent may be a taxoid, or that the antibody is a 4D5 anti-Her2 antibody (which is an antibody that binds to the extracellular domain of HER2). The claims do not specifically recite a dosage. However, a dosage for an anti-4D5 Her2 antibody was known in the art as evidenced by the teachings of Baselga-1996. The claims do not specifically recite that the chemotherapeutic agent may be a taxoid, or that it be in combination with a further growth inhibitory agent. However, the use of taxoids and a further growth inhibiting agent such as carboplatin for the treatment of breast cancer is known in the prior art as evidenced by the

teachings of either Perez, and the combination of a 4D5 anti-HER2 antibody and paclitaxel (a taxoid) exhibits synergism in reducing the growth of xenograft tumors in nude mice, as taught by Baselga-1994a. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have used a taxoid such as paclitaxel in the treatment methods of claims 1-7 and 9-13 of US Patent No. 6,387,371 to make the claimed methods.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anne Holleran, whose telephone number is (571) 272-0833. The examiner can normally be reached on Monday through Friday from 9:30 am to 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Larry Helms, can be reached on (571) 272-0832. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (571) 272-1600.

Papers related to this application may be submitted to Group 1600 by facsimile transmission. The faxing of such papers must conform to the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The Official Fax number for Group 1600 is (571) 273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications

may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

Anne L. Holleran
Patent Examiner
June 23, 2008
/Alana M. Harris, Ph.D./
Primary Examiner, Art Unit 1643

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	10356824	PATON ET AL.
	Examiner	Art Unit
	ANNE L HOLLERAN	1643

SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
updated search of US Patents, EAST, history in file	6/23/2008	alh

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	237	(her2 or erbb2 or cerbb2 or erbb2her2 or cerbb2her2 or p185 or p185her2 or p185erbb2 or p185cerbb2 or p185her2cerbb2 or p185her2erbb2 or p185erbb2her2).clm.	USPAT	OR	OFF	2008/06/23 11:36
L2	2673	424/130.1,133.1,138.1,141.1,143.1,155.1,174.1.ccls.	USPAT	OR	OFF	2008/06/23 11:36
L3	39	1 and 2	USPAT	OR	OFF	2008/06/23 11:36

6/23/2008 11:37:10 AM
C:\Documents and Settings\AHolleran\My Documents\EAST\Workspaces\10356824.wsp

0 BLAST results enclosed:

The undersigned also wishes to bring to the attention of the Examiner BLAST results of computerized alignments of the against sequences contained in the nucleotide and protein databases. The BLAST results are provided in paper form and are identified as reference "BLAST Results A-1- A-0" (nucleotide) and "BLAST Results B-1 - B-0" (protein) on the PTO Form 1449. Applicant requests that these references also be considered and that the Form 1449 be initialed to indicate the Examiner's consideration of the references.

A concise explanation of relevance of the items listed on PTO-1449 is:

(X) not given

0 given for each listed item

0 given for non-English language listed item(s) (Required)

0 in the form of an English language copy of a Search Report from a foreign patent office, issued in a counterpart application, which refers to the relevant portions of the references.

In accordance with 37 CFR §1.97(g), the filing of this information disclosure statement shall not be construed as a representation that a search has been made.

In accordance with 37 CFR §1.97(h), the filing of this information disclosure statement shall not be construed to be an admission that the information cited in the statement is, or is considered to be, material to patentability as defined in 37 CFR § 1.56(b).

The Commissioner is hereby authorized to charge any additional fees required under 37 CFR 1.16 and 1.17 for this Information Disclosure Statement, or credit overpayment to Deposit Account No. 07-0630.

Respectfully submitted,

GENENTECH, INC.

Date: February 8, 2008

By: /Wendy M. Lee/
Wendy M. Lee
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Telephone No. (650) 225-1994

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /ALH/

FORM PTO-1449 U.S. Dept. of Commerce Patent and Trademark Office

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P1256R1D1	10/356,824
Applicant: Hellmann et al.	
Filing Date: 03 Feb 2003	Group: 1643

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
/ALH/	*275	2001/0014326 A1	16.08.01	Andya et al.			
	*276	2002/0001587 A1	03.01.02	Erickson et al.			
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Examiner /Anne Holleran/ (06/16/2008) Date Considered

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /ALH/

FORM PTO-1449	U.S. Dept. of Commerce Patent and Trademark Office	Atty Docket No. P1256R1D1	Serial No. 10/356,824
LIST OF DISCLOSURES CITED BY APPLICANT (Use several sheets if necessary)		Applicant Hellmann et al.	
		Filing Date 03 Feb 2003	Group 1643

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Examiner	/Anne Holleran/ (06/16/2008)	Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /ALH/

USCOMM-DC 80-398.

FORM PTO-1449	U.S. Dept. of Commerce Patent and Trademark Office	Atty Docket No. P1256R1D1	Serial No. 10/356,824
LIST OF DISCLOSURES CITED BY APPLICANT (Use several sheets if necessary)		Applicant Hellmann et al.	
		Filing Date 03 Feb 2003	Group 1643

OTHER DISCLOSURES (Including Author, Title, Date, Pertinent Pages, etc.)

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Examiner	/Anne Holleran/ (06/16/2008)	Date Considered	

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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /ALH/

USCOMM-DC 80-398

PTO/SB/80 (01-06)
Approved for use through 12/31/2008. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(b).

I hereby appoint:

☑ Practitioners associated with the Customer Number: 35489

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

as attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(b).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(b) to:

☑ The address associated with Customer Number: 35489

OR

☐ Firm or Individual Name			
Address			
City		State	Zip
Country			
Telephone		Email	

Assignee Name and Address:

Genentech, Inc. (a corporation)
1 DNA Way
South San Francisco, CA 94080

A copy of this form, together with a statement under 37 CFR 3.73(b) (Form PTO/SB/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(b) may be completed by one of the practitioners appointed in this form if the appointed practitioner is authorized to act on behalf of the assignee, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature		Date	November 25, 2008
Name	Mark Kresnak	Telephone	(650) 225-4461
Title	Associate General Counsel, Director		

AZ 11.25.08

PTO/SB/96 (10-08)
Approved for use through 11/30/2008. OMB 0651-0031
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STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: GENENTECH, INC.

Application No./Patent No.: 10/356,824 Filed/Issue Date: February 3, 2003

Entitled: TREATMENT WITH ANTI-ERBB2 ANTIBODIES

Genentech, Inc. (Name of Assignee), a Corporation (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. [✓] the assignee of the entire right, title, and interest; or
2. [ ] an assignee of less than the entire right, title and interest
(The extent (by percentage) of its ownership interest is _____ %)

in the patent application/patent identified above by virtue of either:

A. [✓] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 013948, Frame 0948, or for which a copy therefore is attached.

OR

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

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The document was recorded in the United States Patent and Trademark Office at
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[ ] Additional documents in the chain of title are listed on a supplemental sheet.

[✓] As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

_____ 12/12/2008
Signature Date

James A. Fox 650-752-3137
Printed or Typed Name Telephone Number

Attorney of Record / Registration No: 38455
Title

Electronic Acknowledgement Receipt

EFS ID:	4446140
Application Number:	10356824
International Application Number:	
Confirmation Number:	4326
Title of Invention:	Treatment with anti-ErbB2 antibodies
First Named Inventor/Applicant Name:	Virginia E. Paton
Customer Number:	09157
Filer:	Ginger R. Dreger/Rossetti Saena
Filer Authorized By:	Ginger R. Dreger
Attorney Docket Number:	P1256R1D1
Receipt Date:	12-DEC-2008
Filing Date:	03-FEB-2003
Time Stamp:	14:32:02
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	POA.pdf	88090 [illegible]	no	2

Warnings:	
Information:	

Total Files Size (in bytes):	88090

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/356,824	02/03/2003	Virginia E. Paton	P1256R1D1

CONFIRMATION NO. 4326

35489
GOODWIN PROCTER LLP
135 COMMONWEALTH DRIVE
MENLO PARK, CA 94025

POA ACCEPTANCE LETTER

OC000000033657152

Date Mailed: 12/19/2008

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 12/12/2008.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/gbien-aime/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/356,824	02/03/2003	Virginia E. Paton	P1256R1D1

CONFIRMATION NO. 4326

9157
GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

POWER OF ATTORNEY NOTICE

OC000000033657118

Date Mailed: 12/19/2008

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 12/12/2008.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

/gbien-aime/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Virginia E. Paton	Attorney Docket #:	GNE-0329R1D1
Serial No.	10/356,824	Group Art Unit	1643
Filing Date	02/03/2003	Examiner:	Holleran, Anne L.
Customer No.:	35489	Confirmation No.:	4326
Title:	**TREATMENT WITH ANTI-ErbB2 ANTIBODIES**		

FILED VIA EFS – December 29, 2008

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

AMENDMENT AND RESPONSE TO OFFICE ACTION

Sir:

This is in response to the Office Action mailed on June 26, 2008 in connection with the above-identified patent application. The present Amendment and Response is accompanied by a Request for a Three-Month Extension of time and is therefore timely.

Amendments to the Claims begin on page 2 of this paper.

Remarks/Arguments begin on page 5 of this paper.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1-33. (Canceled)

34. (Previously presented) A method for the treatment of a human patient with breast cancer that overexpresses ErbB2 receptor, comprising administering a combination of an antibody that binds ErbB2, a taxoid, and a further growth inhibitory agent to the human patient in an amount effective to extend the time to disease progression in the human patient, wherein the antibody binds to epitope 4D5 within the ErbB2 extracellular domain sequence.

35. (Previously presented) The method of claim 34 wherein the antibody if a humanixed 4D5 anti-ErbB2 antibody.

36. (Previously presented) The method of claim 34 wherein the antibody cross-blocks binding of 4D5 to the ErbB2 extracellular domain sequence.

37. (Previously presented) The method of claim 34 wherein the antibody binds to amino acid residues in the region from about residue 529 to about residue 625 of the ErbB2 extracellular domain sequence.

38. (Previously presented) A method for the treatment o f a human patient with breast cancer characterized by overexpression of ErbB2 receptor, comprising administering an effective amount of a combination of an anti-ErbB2 antibody which binds epitope 4D5 within the ErbB2 extracellular domain sequence, a taxoid, and a further therapeutic agent, to the human patient.

39. (Previously presented) The method of claim 38 wherein the breast cancer is metastatic breast carcinoma.

40-41. (Canceled)

42. (Previously presented) The method of claim 38 wherein the antibody is a humanized 4D5 anti-ErbB2 antibody.

43. (Previously presented) The method of claim 38 wherein the taxoid is paclitaxel or docetaxel.

44. (Previously presented) The method of claim 38 wherein efficacy is measured by determining the time to disease progression or the response rate.

45-46. (Canceled)

47. (Previously presented) The method of claim 38, wherein the further therapeutic agent is selected from the group consisting of: another ErbB2 antibody, EGFR antibody, ErbB3 antibody, ErbB4 antibody, vascular endothelial growth factor (VEGF) antibody, cytokine, and growth inhibitory agent.

48. (Previously presented) A method for the treatment of a human patient with ErbB2 overexpressing breast cancer, comprising administering a combination of an antibody that binds epitope 4D5 within the ErbB2 extracellular domain sequence, a taxoid and a further growth inhibitory agent, in the absence of an anthracycline derivative, to the human patient in an amount effective to extend the time to disease progression in the human patient.

49. (Previously presented) The method of claim 48 wherein the breast cancer is metastatic breast carcinoma.

50. (Withdrawn) The method of claim 38 wherein the third therapeutic agent is another ErbB2 antibody.

51. (Withdrawn) The method of claim 38 wherein the third therapeutic agent is a vascular endothelial growth factor (VEGF) antibody.

52. (Currently amended) The method of claim 38 wherein the ~~third~~ further therapeutic agent is a growth inhibitory agent.

53. (Previously presented) The method of claim 52 wherein the growth inhibitory agent is a DNA alkylating agent.