The present invention concerns the treatment of disorders characterized by the overexpression of ErbB2. More specifically, the invention concerns the treatment of human patients susceptible to or diagnosed with cancer overexpressing ErbB2 with anti-ErbB2 antibody.
when administered every 3 weeks to women with metastatic breast cancer: preliminary results of a phase II trial” European Journal of Cancer (2002).


Baselga et al., “Monoclonal antibodies directed against growth factor receptors enhance the efficacy of chemotherapeutic agents” Annals of Oncology (abstract #010) S(Suppl. 5) (1994).


D’Fiore et al., “erbB-2 is a potent oncogene when overexpressed in NIH/3T3 cells” Science 237(4811):178-182 (Jul. 10, 1987).


Yamamoto et al., “Similarity of protein encoded by the human c-erbB–2 gene to epidermal growth factor receptor” (GenBank accession number X03363) (Mar. 30, 1995).


* cited by examiner
3H4 epitope (SEQ ID NO:8) 58 residues
VEECRVLQGLPREYVNARHCLPCHPECQPGNSVTCFGPEADQCVACAHYKDPFPCVAR

4D5 epitope (SEQ ID NO:9) 64 residues
LPCHPECQPGNSVTCFGPEADQCVACAHYKDPFPCVARCPSGVKPDLSYMIPWFKFDEEGACQP

FIG._1

1  MelAALCRWGLLALALLFPGAAGSTQVCTGTDNLRLPA
38  SPTHELMLRLHLYQGCQVQGNETLYPTNASLSFL
75  ODIOPVQGGVLIADNHQURVQVPLRRLRVPTQLPQDN
112  YALAVLDNGDFLNTTPVTGASPGGLRELQSLTEI
149  LEKVVLQQRNPOLCYQDTILMKDIFPHKNQLALTLD
186  TNRSRA

FIG._2
Cell Mean for TUMOR VOL (mm³)

- E25.4 mg/kg IV
- IV.1 mg/kg anti-HER2
- IV.2 mg/kg anti-HER2
- IV.4 mg/kg anti-HER2
- SC.2 mg/kg anti-HER2

FIG. 4A
Cell Mean for TUMOR VOl. (mm³)

- E25.4 mg/kg, IV
- IV.2 mg/kg, anti-HER2
- IV.4 mg/kg, anti-HER2
- SC.2 mg/kg, anti-HER2

FIG. 4B
VARIABLE LIGHT

1 10 20 30 40

2C4 DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIIGVA----] WYQQRP

574 DIQMTQSPSSLSASVGDRVTITC [KASQDVSIIGVA----] WYQQKP

hum kI DIQMTQSPSSLSASVGDRVTITC [RASQSVSTSSYSYM] WYQQKP

50 60 70 80

2C4 GQSKKLLIIY [ASRYST] GVPDRFTGSGGTGDTFTISSVQA

574 GKAPKLLIIY [ASRYST] GVPSRFSGSGGTGDTFTLTISSLQP

hum kI GKAPKLLIIY [AASSLES] GVPSRFSGSGGTGDTFTLTISSLQP

FIG._5A

VARIABLE HEAVY

1 10 20 30 40

2C4 EVQLQSQSPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS

574 EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA

hum III EVQLVESGGGSVQPGSLRLSCAAS [GFTFSSYAMS] WVRQA

50 60 70 80

2C4 HGKSLEWIG [DVNPNSGGSYINQFRKG] KASLTVDRSSRIVYM

574 PGKGLEWVA [DVNPNSGGSYINQFRKG] RPTLSVDRSNTLYL

hum III PGKGLEWVS [VISGDGGSTYYADSVKG] RPTISRDDSNTLYL

90 100 110

2C4 ELRSSTFEDTAVVYCAR [NLGPSFYFDY] WGGQGTLTVSS (SEQ ID NO:11)

574 QMNSLRAEYDTEYIYCAR [NLGPSFYFDY] WGGQTLTVSS (SEQ ID NO:13)

hum III QMNSLRAEYDTEYIYCAR [GRGGGS--DY] WGGQTLTVSS (SEQ ID NO:15)

FIG._5B
1 DOSAGES FOR TREATMENT WITH ANTI-ERBB2 ANTIBODIES

RELATED APPLICATIONS

This application is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application No. 60/151,018, filed Aug. 27, 1999 and No. 60/213,822, filed Jun. 23, 2000, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention concerns the treatment of disorders characterized by the overexpression of ErbB2 or disorders expressing epidermal growth factor receptor (EGFR), comprising administering to a human or animal presenting the disorders a therapeutically effective amount of an antibody that binds ErbB2. More specifically, the invention concerns the treatment of human patients susceptible to or diagnosed with cancer overexpressing ErbB2 or expressing EGFR, where the treatment is with an anti-ErbB2 antibody administered by front loading the dose of antibody during treatment by intravenous and/or subcutaneous administration. The invention optionally includes treatment of cancer in a human patient with a combination of an anti-ErbB2 antibody and a chemotherapeutic agent, such as, but not limited to, a taxoid. The taxoid may be, but is not limited to paclitaxel or docetaxel. The invention further includes treatment of cancer in a human patient with a combination of anti-ErbB2 antibody and a chemotherapeutic agent, such as, but not limited to, an anthracycline derivative. Optionally, treatment with a combination of anti-ErbB2 and an anthracycline derivative includes treatment with an effective amount of a cardioprotectant. The present invention further concerns infrequent dosing of 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 ErbB2 gene (erbB2, also known as her2, or c-erbB-2), which encodes a 185-kd transmembrane glycoprotein receptor (p185HER2) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer. A number of 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]).

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 products and proteins encoded by the rat equivalent of the erbB2 gene (neu) have been described. Drebìen et al. (Proc. Natl. Acad. Sci. USA 84:7159–7163 [1987]) and Drebìen 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-l-l cells (NIH-3T3 cells transformed with the neu proto-oncogene) which inhibits colony formation of these cells. In Drebìen 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. Drebìen 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. Drebìen 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 more detail in the reference by Hudziak et al., Mol. Cell. Biol. 9(3):1165–1172 (1989) which 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-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also WO98/06692 published Jul. 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); Vitieta 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’ouzca et al. Proc. Natl. Acad. Sci. 91:7202–7206 (1994).

Tagliafure 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 5:543–548 (1989) generated a panel of anti-ErbB2 antibodies with varying epitope specificities, including the antibody designated TAl. This TAl antibody was found to induce accelerated endocytosis of ErbB2 (see Maier et al. Cancer Res. 51:5361–5369 [1991]). Baccus et al. Molecular Caeroinogenses 3:350–362 (1990) report that the TAl antibody induced maturation of the breast cancer cell lines AU-565 (which overexpresses the erbB2 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 fibrosarcoma transformed by overexpression of the erbB2 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-mediated cell-dependent 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 fibrosarcoma 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.


A recombinant humanized anti-ErbB2 monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2, HERCEPTIN®, or HERCEPTIN®-anti-ErbB2 antibody) has been clinically active in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior anti-cancer therapy (Bagel et al., J. Clin. Oncol. 14:737–744 [1996]). The recommended initial loading dose for HERCEPTIN® is 4 mg/kg administered as a 90-minute infusion. The recommended weekly maintenance dose is 2 mg/kg and can be administered as a 30-minute infusion if the initial loading dose is well tolerated.

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 Charney, 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 chemotherapy regimens, including CMF (cyclophosphamide, methotrexate, and fluorouracil) 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

The present invention concerns the discovery that an early attainment of an efficacious target trough serum concentration by providing an initial dose or doses of anti-ErbB2 antibodies followed by subsequent doses of equal or smaller amounts of antibody (greater front loading) is more efficacious than conventional treatments. The efficacious target trough serum concentration is reached in 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, and most preferably 1 week or less, including 1 day or less. The target serum concentration is thereafter maintained by the administration of maintenance doses of equal or smaller amounts for the remainder of the treatment regimen or until suppression of disease symptoms is achieved.

The invention further concerns a method for the treatment of a human patient susceptible to or diagnosed with a disorder characterized by overexpression of ErbB2 receptor comprising administering a therapeutically effective amount of an anti-ErbB2 antibody subcutaneously. Preferably, the initial dose (or doses) as well as the subsequent maintenance dose or doses are administered subcutaneously. Optionally, where the patient’s tolerance to the anti-ErbB2 antibody is unknown, the initial dose is administered by intravenous infusion, followed by subcutaneous administration of the maintenance doses if the patient’s tolerance for the antibody is acceptable.

According to the invention, the method of treatment involves administration of an initial dose of anti-ErbB2 antibody of more than approximately 4 mg/kg, preferably more than approximately 5 mg/kg. The maximum initial dose or a subsequent dose does not exceed 50 mg/kg, preferably does not exceed 40 mg/kg, and more preferably does not exceed 30 mg/kg. Administration is by intravenous or subcutaneous administration, preferably intravenous infusion or bolus injection, or more preferably subcutaneous bolus injection. The initial dose may be one or more administrations of drug sufficient to reach the target trough serum concentration in 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, and most preferably 1 week or less, including one day or less.

According to the invention, the initial dose or doses is/are followed by subsequent doses of equal or smaller amounts of antibody at intervals sufficiently close to maintain the trough serum concentration of antibody at or above an efficacious target level. Preferably, an initial dose or subsequent dose does not exceed 50 mg/kg, and each subsequent dose is at least 0.01 mg/kg. Preferably the amount of drug
administered is sufficient to maintain the target trough serum concentration such that the interval between administration cycles is at least one week. Preferably the trough serum concentration does not exceed 2500 μg/ml and does not fall below 0.01 mg/ml during treatment. The front loading drug treatment method of the invention has the advantage of increased efficacy by reaching a target serum drug concentration early in treatment. The subcutaneous delivery of maintenance doses according to the invention has the advantage of being convenient for the patient and health care professionals, reducing time and costs for drug treatment. Preferably, the initial dose (or the last dose within an initial dose series) is separated in time from the first subsequent dose by 4 weeks or less, preferably 3 weeks or less, more preferably 3 weeks or less, most preferably 1 week or less.

In an embodiment of the invention, the initial dose of anti-ErbB2 is 6 mg/kg, 8 mg/kg, or 12 mg/kg delivered by intravenous or subcutaneous administration, such as intravenous infusion or subcutaneous bolus injection. The subsequent maintenance doses are 2 mg/kg delivered once per week by intravenous infusion, intravenous bolus injection, subcutaneous infusion, or subcutaneous bolus injection. The choice of delivery method for the initial and maintenance doses is made according to the ability of the animal or human patient to tolerate introduction of the antibody into the body. Where the antibody is well-tolerated, the time of infusion may be reduced. The choice of delivery method as disclosed for this embodiment applies to all drug delivery regimens contemplated according to the invention.

In another embodiment, the invention includes an initial dose of 12 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 6 mg/kg once per 3 weeks.

In still another embodiment, the invention includes an initial dose of 8 mg/kg anti-ErbB2 antibody, followed by 6 mg/kg once per 3 weeks.

In yet another embodiment, the invention includes an initial dose of 8 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 6 mg/kg once per week or 8 mg/kg once every 2 to 3 weeks.

In another embodiment, the invention includes initial doses of at least 1 mg/kg, preferably 4 mg/kg, anti-ErbB2 antibody on each of days 1, 2 and 3, followed by subsequent maintenance doses of 6 mg/kg once per 3 weeks.

In another embodiment, the invention includes an initial dose of 4 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 2 mg/kg twice per week, wherein the maintenance doses are separated by 3 days.

In still another embodiment, the invention includes a cycle of dosing in which delivery of anti-ErbB2 antibody is 2-3 times per week for 3 weeks. In one embodiment of the invention, each dose is approximately 25 mg/kg or less for a human patient, preferably approximately 10 mg/kg or less. This 3 week cycle is preferably repeated as necessary to achieve suppression of disease symptoms.

In another embodiment, the invention includes a cycle of dosing in which delivery of anti-ErbB2 antibody is daily for 5 days. According to the invention, the cycle is preferably repeated as necessary to achieve suppression of disease symptoms.

The disorder preferably is a benign or malignant tumor characterized by the overexpression of the ErbB2 receptor, e.g. a cancer, such as, breast cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer. The method of the invention may further comprise administration of a chemotherapeutic agent other than an anthracycline, e.g. doxorubicin or epirubicin. The chemotherapeutic agent preferably is a taxoid, such as TAXOL® (paclitaxel) or a TAXOL® derivative.

Preferred anti-ErbB2 antibodies bind the extracellular domain of the ErbB2 receptor, and preferably bind to the epitope 4D5 or 3H4 within the ErbB2 extracellular domain sequence. More preferably, the antibody is the antibody 4D5, most preferably in a humanized form. Other preferred ErbB2-binding antibodies include, but are not limited to, antibodies 7C2, 7F3, and 2C4, preferably in a humanized form.

The method of the present invention is particularly suitable for the treatment of breast or ovarian cancer, characterized by the overexpression of the ErbB2 receptor.

The present application also provides a method of therapy involving infrequent dosing of an anti-ErbB2 antibody. In particular, the invention provides a method for the treatment of cancer (e.g. cancer characterized by overexpression of the ErbB2 receptor) in a human patient comprising administering to the patient a first dose of an anti-ErbB2 antibody followed by at least one subsequent dose of the antibody, wherein the first dose and subsequent dose are separated from each other in time by at least about two weeks (e.g. from about two weeks to about two months), and optionally at least about three weeks (e.g. from about three weeks to about six weeks). For instance, the antibody may be administered every three weeks, about two to about 20 times, e.g. about six times. The first dose and subsequent dose may each be from about 2 mg/kg to about 16 mg/kg; e.g. from about 4 mg/kg to about 12 mg/kg; and optionally from about 6 mg/kg to about 12 mg/kg.

In another embodiment, the invention includes an initial dose of about 4 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of about 2 mg/kg twice per week, wherein the maintenance doses are separated by 3 days.

The presently described dosing protocols may be applied to other anti-ErbB antibodies such as anti-epidermal growth factor receptor (EGFR), anti-ErbB3 and anti-ErbB4 antibodies. Thus, the invention provides a method for the treatment of cancer in a human patient, comprising administering an effective amount of an anti-ErbB antibody to the human patient, the method comprising administering to the patient an initial dose of at least approximately 5 mg/kg of the anti-ErbB antibody; and administering to the patient a plurality of subsequent doses of the antibody in an amount that is approximately the same or less than the initial dose. Alternatively, or additionally, the invention pertains to a method for the treatment of cancer in a human patient comprising administering to the patient a first dose of an anti-ErbB antibody followed by at least one subsequent dose of the antibody, wherein the first dose and subsequent dose are separated from each other in time by at least about two weeks.
7 weeks. The invention additionally provides an article of manufacture, comprising a container, a composition within the container comprising an anti-ErbB2 antibody, and a package insert containing instructions to administer the antibody according to such methods.

In another aspect, the invention concerns an article of manufacture, comprising a container, a composition within the container comprising an anti-ErbB2 antibody, optionally a chemotherapeutic agent, and a package insert further includes instructions to administer the anti-ErbB2 antibody at an initial dose of 5 mg/kg followed by the same or smaller subsequent dose or doses. In another embodiment of the invention, the package insert further includes instructions to administer the anti-ErbB2 antibody subcutaneously for at least one of the doses, preferably for all of the subsequent doses following the initial dose, most preferably for all doses.

In a further aspect, the invention provides a method of treating ErbB2-expressing cancer in a human patient comprising administering to the patient effective amounts of an anti-ErbB2 antibody and a chemotherapeutic agent. In one embodiment of the invention, the chemotherapeutic agent is a taxoid, such as TAXOL®. In another embodiment, the chemotherapeutic agent is an anthracycline, including but not limited to, doxorubicin or epirubicin. In still another embodiment of the invention, treatment with an anti-ErbB2 antibody and an anthracycline-type chemotherapeutics in combination with a cardioprotectant to the patient. In still another embodiment, an anthracycline derivative is not administered to the patient with the anti-ErbB2 antibody. One or more additional chemotherapeutic agents may also be administered to the patient. The cancer is preferably characterized by overexpression of ErbB2.

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 administer the anti-ErbB2 antibody according to such methods.

The methods and compositions of the invention comprise an anti-ErbB2 antibody and include a humanized anti-ErbB2 antibody. Thus, the invention further pertains to a composition comprising an antibody that binds ErbB2 and the use of the antibody for treating ErbB2 expressing cancer, e.g., ErbB2 overexpressing cancer, in a human. The invention also pertains to the use of the antibody for treating EGFR expressing cancer. Preferably the antibody is a monoclonal antibody 4D5, e.g., humanized 4D5 (and preferably huMAb4D5-S (HERCEPTIN®-anti-ErbB2 antibody); or monoclonal antibody 2C4, e.g., humanized 2C4. The antibody may be an intact antibody (e.g., an intact IgG, antibody) or an antibody fragment (e.g., a Fab, (Fab')2, diabody, and the like). The variable light chain and variable heavy chain regions of humanized anti-ErbB2 antibody 2C4 are shown in FIGS. 5A and 5B.

**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 [October 1993]; Renz et al. J. Cell Biol. 126(6):1395–1406 [June 1994]). The anti-proliferative MAb s 4D5 and 3H4 bind adjacent to the transmembrane domain. The various ErbB2-EC domain 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 35S methionine and 35S 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 electrophorized onto a membrane and analyzed by autoradiography. SEQ ID NOs:8 and 9 depict the 3H4 and 4D5 epitopes, respectively.
An “ErbB receptor” is a receptor protein tyrosine kinase which belongs to the ErbB receptor family and includes EGFR, HER2, ErbB3 and ErbB4 receptors as well as TEGFR (U.S. Pat. No. 5,708,156) and other members of this family to be identified in the future. The ErbB receptor will generally comprise an extracellular domain, which may bind an ErbB ligand; a lipophilic transmembrane domain; a conserved intracellular tyrosine kinase domain; and a carboxyl-terminal signaling domain harboring several tyrosine residues which can be phosphorylated. The ErbB receptor may be a native sequence ErbB receptor or an amino acid sequence variant thereof. Preferably the ErbB receptor is native sequence human ErbB receptor.

The terms “ErbB1,” “epidermal growth factor receptor” and “EGFR” are used interchangeably herein and refer to native sequence EGFR as disclosed, for example, in Carpenter et al. 

Appln No 599,274; Plowman et al., which bind HER3 are described in U.S. Pat. No. 5,968,511 which belongs to the ErbB receptor family and includes “c-Erb-B2” and “HER2” when used herein refer to the 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

The terms “ErbB2” and “HER2” generally comprise an extracellular domain, which may bind to an ErbB ligand; a lipophilic transmembrane domain; a conserved intracellular tyrosine kinase domain; and a carboxyl-terminal signaling domain harboring several tyrosine residues which can be phosphorylated. The ErbB receptor may be a native sequence ErbB receptor or an amino acid sequence variant thereof. Preferably the ErbB receptor is native sequence human ErbB receptor.

The terms “ErbB2”, “c-ErbB2” and “HER2” are used interchangeably herein and refer to native sequence EGFR as disclosed, for example, in PNAS (USA) 86:9193-9197 (1989), including variants thereof. Examples of antibodies which bind HER2 are described in U.S. Pat. No. 5,308,511 (Akita and Sliwkowski), e.g. the 8B8 antibody (ATCC CRL 12070) or a humanized variant thereof.

The terms “ErbB4” and “HER4” herein refer to the receptor polypeptide as disclosed, for example, in U.S. Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus et al. PNAS (USA) 86:1746-1750 (1993); and Plowman et al., Nature, 365:473-475 (1993), including variants thereof as described in the example herein; and nuclear/chromatin condensation along with DNA fragmentation. 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 SKOV V 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 (37F3 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 erbB1, erbB3 and/or erbB4 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).

“Herregulin” (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 herregulin polypeptides encompassed by this term are disclosed in Holmes et al., Science, 256:1200–1210 (1992); WO 92/20798; Wen et al., Mol. Cell. Biol., 14(3):1909–1919 (1994); 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(1909–1919)).

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 Sliwowski 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 constant domain 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 (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) 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 FRs 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 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.

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 ability 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.

“Fc” 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 Vγ-Vδ dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv containing 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. Fab’(α)’ 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 (k) 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, IgA1, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ1, γ2, 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 (Zapatero 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 being obtained from a substantially homogeneous antibody preparation 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. Pat. No. 4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson et al., Nature, 352: 624–628 (1991) and Marks et al., J. Mol. Biol., 222: 581–597 (1991), for example.

The monoclonal antibodies herein specifically include “chimeric” antibodies (immunoglobulins) in which portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; 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, 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 CDRs 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); Reichmann et al., Nature, 332: 323–329 (1988); and Presta, Curr. Op. Struct. Biol., 2: 593–596 (1992). The humanized antibody includes a PRI-MATIZED™ 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, Rosenberg 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 is 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 sequencer, 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 Fe region of an IgG molecule (e.g., IgG1, IgG2, IgG3, or IgG4) 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, stomatal and glactosalic, cervical, ovarian, liver, cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial epithelial, stromal and blastocoelic disorders; and chronic and acute disorders or diseases including those characterised by unregulated cell growth. Examples of such cancers include squamous cell cancer, small-cell cancer, pancreatic cancer, glisteningoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of 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. 131I, 125I, 89Sr and 186Re), 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 thiopeta and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboxaquone, mertaclopa, and uredopa; ethylenimines and methylmelamines including altretamine, triethylene-melamine, triethylenephosphoramide, triethyl-eneethiophosphoramide and trimethylolomelamine; nitrodermamines such as altretamine, bestrabucil, bisantrene, edatraxate, defoamone, demecolcine, diaziquone, elfornithine, elliptinium acetate, etoglucid; gallium nitrate; hydroxyurea; lentinan; lomustine; mitoguazone; mitoxantrone; nimustine; ranimustine; melphan; novembichin, phenesterine, prednimustine.

Efficacy can be measured in conventional ways, depending on the condition to be treated. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP), or determining the response rates (RR) (see Example 1, below). Therapeutically effective amount also refers to a target serum concentration, such as a trough serum concentration, that has been shown to be effective in suppressing disease symptoms when maintained for a period of time.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glisteningoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of 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. 131I, 125I, 89Sr and 186Re), 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 thiopeta and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboxaquone, mertaclopa, and uredopa; ethylenimines and methylmelamines including altretamine, triethylene-melamine, triethylenephosphoramide, triethyl-eneethiophosphoramide and trimethylolomelamine; nitrodermamines such as altretamine, bestrabucil, bisantrene, edatraxate, defoamone, demecolcine, diaziquone, elfornithine, elliptinium acetate, etoglucid; gallium nitrate; hydroxyurea; lentinan; lomustine; mitoguazone; mitoxantrone; nimustine; ranimustine; melphan; novembichin, phenesterine, prednimustine.
Drug delivery, prodrug form for use in this invention include, but are not limited to, polyacrylamides, polystyrene, polyvinyl alcohol and silylphenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 50 substance that is less cytotoxic to tumor cells compared to 35 sequence cytokines.

Solid phase of discrete particles, such as those described in patents: 5-fiuorocytosine and other 5-fiuorouridine prodrugs which can be converted into the more active cytotoxic free drug. 

Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), controlled porosity silica and silylcones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in other instances it is a purification column (e.g., affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Pat. No. 4,275,149.

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 antibody) 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, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

The term “serum concentration,” “serum drug concentration,” or “serum HERCEPTIN® anti-ErbB2 antibody concentration” refers to the concentration of a drug, such as HERCEPTIN® anti-ErbB2 antibody, in the blood serum of an animal or human patient being treated with the drug. Serum concentration of HERCEPTIN® anti-ErbB2 antibody, for example, is preferably determined by immunoassay. Preferably, the immunoassay is an ELISA according to the procedure disclosed herein.

The term “trough serum concentration” refers to the minimal serum drug concentration shortly after delivery of the drug into the animal or human patient, after the drug has been distributed throughout the blood system, but before significant tissue distribution, metabolism or excretion of drug by the body has occurred.

The term “trough serum concentration” refers to the serum drug concentration at a time after delivery of a previous dose and immediately prior to delivery of the next subsequent dose of drug in a series of doses. Generally, the trough serum concentration is a minimum sustained efficacious drug concentration in the series of drug administrations. Also, the trough serum concentration is frequently targeted as a minimum serum concentration for efficacy because it represents the serum concentration at which another dose of drug is to be administered as part of the treatment regimen. If the delivery of drug is by intravenous administration, the trough serum concentration is most preferably attained within 1 day of a front loading initial intravenous delivery. If the delivery of drug is by subcutaneous administration, the peak serum concentration is preferably attained in 3 days or less. According to the invention, the trough serum concentration is preferably attained in 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, most preferably in 1 week or less, including 1 day or less using any of the drug delivery methods disclosed herein.

The term “intravenous infusion” refers to introduction of a drug into the vein of an animal or human patient over a period of time greater than approximately 5 minutes, preferably between approximately 30 to 90 minutes, although, according to the invention, intravenous infusion is alternatively administered for 10 hours or less.

The term “intravenous bolus” or “intravenous push” refers to drug administration into a vein of an animal or human such that the body receives the drug in approximately 15 minutes or less, preferably 5 minutes or less.

The term “subcutaneous administration” refers to introduction of a drug under the skin of an animal or human patient, preferably within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle. The pocket may be created by pinching or drawing the skin up and away from underlying tissue.

The term “subcutaneous infusion” refers to introduction of a drug under the skin of an animal or human patient,
preferably within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle for a period of time including, but not limited to, 30 minutes or less, or 90 minutes or less. Optionally, the infusion may be made by subcutaneous implantation of a drug delivery pump implanted under the skin of the animal or human patient, wherein the pump delivers a predetermined amount of drug for a predetermined period of time, such as 30 minutes, 90 minutes, or a time period spanning the length of the treatment regimen.

The term “subcutaneous bolus” refers to drug administration beneath the skin of an animal or human patient, where bolus drug delivery is preferably less than approximately 15 minutes, more preferably less than 5 minutes, and most preferably less than 60 seconds. Administration is preferably within a pocket between the skin and underlying tissue, where the pocket is created, for example, by pinching or drawing the skin up and away from underlying tissue.

The term “front loading” when referring to drug administration is meant to describe an initially higher dose followed by the same or lower doses at intervals. The initial higher dose or doses are meant to more rapidly increase the animal or human patient’s serum drug concentration to an efficacious target serum concentration. According to the present invention, front loading is achieved by an initial dose or doses delivered over three weeks or less that causes the animal’s or patient’s serum concentration to reach a target serum trough concentration. Preferably, the initial front loading dose or series of doses is administered within two weeks or less, more preferably in 1 week or less, including 1 day or less. Most preferably, where the initial dose is a single dose and is not followed by a subsequent maintenance dose for at least 1 week, the initial dose is administered in 1 day or less. Where the initial dose is a series of doses, each dose is separated by at least 3 hours, but not more than 3 weeks or less, preferably 2 weeks or less, more preferably 1 week or less, most preferably 1 day or less. To avoid adverse immune reaction to an antibody drug such as an anti-ErbB2 antibody (e.g., HERCEPTIN® anti-ErbB2 antibody) in an animal or patient who has not previously been treated with the antibody, it may be preferable to deliver initial doses of the antibody by intravenous infusion. The present invention includes front loading drug delivery of initial and maintenance doses by infusion or bolus administration, intravenously or subcutaneously.


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 Stanescovski 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, maleimidobenzyal sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, or R'RN=C=NR, where R and R' 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 ½ to ½ the original amount of peptide or conjugate in Freund’s complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal Antibodies

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

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

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

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.
Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, Rockville, Md. USA. Human myeloma and mouse-human heteromyloma 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 in vitro binding assay, such as radioluminimun assay (RLA) 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-M EM 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, hydroxyapatite, 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 Püchthun, Immunol. Revs., 130:151–188 [1992].


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. Pat. No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851 [1984]), or by covalently joining to it 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]; Verhoeven 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. Pat. No. 4,816,567) wherein substantially less than an entire human variable domain has been substituted by the corresponding sequence from a non-human species.

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]; Verhoeven 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. Pat. No. 4,816,567) wherein substantially less than an entire 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 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 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 homologous deletion of the antibody heavy-chain joining region (JH) 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., BioTechnology 10:163–167 [1992]). According to another approach, F(ab')2 fragments can be isolated directly from a 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 I 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 (Milstein 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 antibody, 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 (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 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 WO 96/27011, 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 Cα3 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
immunotherapy of tumor cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/00373, 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. Pat. 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. Slabaly et al., J. Exp. Med., 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody (Fab')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. Kostalny 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 (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VH and VL domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (scFv) 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). (v) 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) with 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 3x10⁶ per dish in 100x20 mm dishes to attach overnight. The medium is then removed and replaced with fresh medium alone or medium containing 10 µg/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, resuspended in 3 ml ice cold Ca²⁺ containing medium, and aliquoted into 35 mm strainer-capped 12x75 tubes (1 ml per tube), 3 tubes per treatment group for removal of cell clumps. Tubes then receive PI (10 µg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce statistically significant levels of cell death as determined by PI uptake are selected.

In order to screen 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 Ca²⁺ binding buffer and aliquoted into tubes as described above for the cell death assay. Tubes then receive labeled annexin (e.g. annexin V-FITC) (1 µg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce statistically significant levels of annexin binding relative to control are selected as apoptosis-inducing antibodies.

In addition to the annexin binding assay, a "DNA staining assay using BT474 cells" is available. In order to perform this assay, BT474 cells which have been treated with the antibody of interest as described in the preceding two paragraphs are incubated with 9µg/ml HOECHST 33342™ for 2 hr at 37° C, then analyzed on an EPICS ELITE™ flow cytometer (Coulter Corporation) using MODFIT™ software (Venty 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 WO 89/06692 is available. According to this assay, SKBR3 cells are grown in a 1:1 mixture of F12 and DME medium supplemented with 10% fetal bovine serum, glutamine and penicillin-streptomycin. The SKBR3 cells are plated at 20,000 cells in a 35 mm cell culture dish
The anti-ErbB2 antibodies disclosed herein may also be formulated as immunoconjugates. 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. Pat. 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). Such 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).

(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 WO 81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Pat. 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 convert 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; protoporphyrin, subtilisin, cathepsins and dianthin proteins, useful for converting prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized with 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 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 phenoxacyethyl or phenylacyetyl 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 functionality active

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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]).

(aii) 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 VH 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 C2 region or VH region, or both, of the antibody fragment.

In one most preferred embodiment, the salvage receptor binding epitope comprises the sequence (5′ to 3′): PKNSMISNTQP (SEQ ID NO:3), and optionally further comprises one or more amino acid residues from one or two loops of the Fc region, or both, of the antibody fragment.

In another most preferred embodiment, the salvage receptor binding epitope is a polypeptide containing the sequence(s) (5′ to 3′): HQNSLGDGK (SEQ ID NO:5), HQNSLDGK (SEQ ID NO:6), or VISSHILGGQ (SEQ ID NO:7); particularly where the antibody fragment is a Fab or Fab′.

In another most preferred embodiment, the salvage receptor binding epitope is a polypeptide containing the sequence(s) (5′ to 3′): HQLNSLDGK (SEQ ID NO:5), HQNSLDGK (SEQ ID NO:6), or VISSHILGGQ (SEQ ID NO:7); and the sequence: PKNSMISNTQP (SEQ ID NO:3).

(xiii) Purification of anti-ErbB2 Antibody

When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter et al., Bio/Technology 10:163–167 [1992] describe a procedure for isolating antibodies which are secreted to the periplasmic space of E. coli. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are preferably first concentrated using a commercially available protein concentration device useful for purification, such as Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibodies may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxyapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Meth. 62:1–13 [1983]). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 [1986]). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose.

Where the antibody comprises a Cγ3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHEROSE™ chromatography on an anion or cation exchange resin (such as a polylysaric acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

When any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5–4.5, preferably performed at low salt concentrations (e.g. from about 0-0.25M salt).

III. Determination of anti-ErbB2 Antibody Concentration in Serum

The following non-limiting assay is useful for determining the presence of and to quantify the amount of specific rhuMAb HER2 (humanized anti-p185HER2 monoclonal antibody, including HERCEPTIN® anti-ErbB2 antibody) in a body fluid of a mammal including, but not limited to, serum, amniotic fluid, milk, umbilical cord serum, ocular aqueous and vitreous liquids, and ocular vitreous gel.

Plate Binding Activity Assay for rhuMAb HER2 (Humanized Anti-p185HER2 Monoclonal Antibody)

The method of assaying rhuMAb HER2 described herein is meant as an example of such a method and is not meant to be limiting. A standardized preparation of rhuMAb HER2 (Genentech, Inc., South San Francisco, Calif.), controls, and serum samples were diluted with Assay Diluent (PBS:0.5% BSA:0.05% Polysorbate 20:0.01% Thimerosal). The dilutions of standardized rhuMAb HER2 were prepared to span a range of concentrations useful for a standard curve. The samples were diluted to fall within the standard curve.
An aliquot of Coat Antigen in Coating buffer (recombinant p185<sup>HER</sup> (Genentech, Inc.) in 0.05 M sodium carbonate buffer) was added to each well of a microtiter plate and incubated at 2–8°C for 12–72 hours. The coating solution was removed and each well was washed six times with water, then blotted to remove excess water.

An aliquot of Assay Diluent was added to each well and incubated for 1–2 hours at ambient temperature with agitation. The wells were washed as in the previous step.

Aliquots of diluted standard, control and sample solutions were added to the wells and incubated at ambient temperature for 1 hour with agitation to allow binding of the antibody to the coating antigen. The wells were washed again with water as in previous steps.

Horse radish peroxidase-conjugate (HRP-conjugate, Goat anti-human IgG Fe conjugated to horseradish peroxidase; Organon Teknika catalog #55253 or equivalent) was diluted with Assay Diluent to yield an appropriate optical density range between the highest and lowest standards. An aliquot of the HRP-conjugate solution was added to each well and incubated at ambient temperature for 1 hour with agitation. The wells were washed with water as in previous steps.

An aliquot of Substrate Solution (o-phenylenediamine (OPD) 5 mg tablet (Sigma P6912 or equivalent) in 12.5 ml 4 mM H<sub>2</sub>O<sub>2</sub> in PBS) was added to each well and incubated for a sufficient period of time (approximately 8–10 minutes) in the dark at ambient temperature to allow color development. The reaction was stopped with an aliquot of 4.5 N sulfuric acid. Optical density was read at 490–492 nm for detection absorbance and 405 nm for reference absorbance.

The standard curve data are plotted and the results for the controls and samples are determined from the standard curve.

### IV. 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 paraarboxamides 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; met al complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as Tween<sup>™</sup>, Pluron<sup>™</sup>, ICS<sup>™</sup> or polyethylene glycol (PEG). Preferred lyophilized anti-ErbB2 antibody formulations are described in WO 97/04801, expressly incorporated herein by reference.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide antibodies which bind to EGFR, ErbB2 (e.g. an antibody which binds a different epitope on ErbB2), ErbB3, ErbB4, or vascular endothelial growth factor (VEGF) in the one formulation. Alternatively, or in addition, 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-(methylmethacrylate) microcapsules, respectively, in colloid al 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 polyelectrolytes, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polyalkylacylenes (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ε ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactide acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>™</sup> (injectable microspheres composed of lactide acid-glycolic acid copolymer and leuprolide acetate), and poly-(D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactide acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins 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 sulhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

### V. Treatment With the Anti-ErbB2 Antibodies

It is contemplated that, according to the present invention, the anti-ErbB2 antibodies may be used to treat various conditions characterized by overexpression and/or activation of the ErbB2 receptor. Exemplary conditions or disorders 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 blastocoele 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, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the antibody is preferred.

The treatment of the present invention involves the administration of an anti-ErbB2 antibody to an animal or human patient, followed at intervals by subsequent doses of equal or smaller doses such that a target serum concentration is achieved and maintained during treatment. Preferably, maintenance doses are delivered by bolus delivery, preferably by subcutaneous bolus administration, making treatment convenient and cost-effective for the patient and health care professionals.

Where combined administration of a chemotherapeutic agent (other than an anthracycline) is desired, the combined administration includes coadministration, using separate formulations or a single pharmacological 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). 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 616 812) 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 growth 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. The ErbB2 antibody may be 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.

In addition to the above therapeutic regimens, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

For the prevention or treatment of disease, the appropriate dosage of anti-ErbB2 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. Where the treatment involves a series of treatments, the initial dose or initial doses are followed at daily or weekly intervals by maintenance doses. Each maintenance dose provides the same or a smaller amount of antibody compared to the amount of antibody administered in the initial dose or doses.

Depending on the type and severity of the disease, about 1 mg/kg to 15 mg/kg (e.g. 0.1–20 mg/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 mg/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. The progress of this therapy is easily monitored by conventional techniques and assays.

According to the invention, dosage regimens may include an initial dose of anti-ErbB2 of 6 mg/kg, 8 mg/kg, or 12 mg/kg delivered by intravenous or subcutaneous infusion, followed by subsequent weekly maintenance doses of 2 mg/kg by intravenous infusion, intravenous bolus injection, subcutaneous infusion, or intramuscular bolus injection. Where the antibody is well-tolerated by the patient, the time of infusion may be reduced.

Alternatively, the invention includes an initial dose of 12 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 6 mg/kg once per 3 weeks.

Another dosage regimen involves an initial dose of 8 mg/kg anti-ErbB2 antibody, followed by 6 mg/kg once per 3 weeks.

Still another dosage regimen involves an initial dose of 8 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 8 mg/kg once per week or 8 mg/kg once every 2 to 3 weeks.

As an alternative regimen, initial doses of 4 mg/kg anti-ErbB2 antibody may be administered on each of days 1, 2 and 3, followed by subsequent maintenance doses of 6 mg/kg once per 3 weeks.

An additional regimen involves an initial dose of 4 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 2 mg/kg twice per week, wherein the maintenance doses are separated by 3 days.

Alternatively, the invention may include a cycle of dosing in which delivery of anti-ErbB2 antibody is 2–3 times per week for 3 weeks. The 3 week cycle is preferably repeated as necessary to achieve suppression of disease symptoms. The invention further includes a cyclic dosage regimen in which delivery of anti-ErbB2 antibody is daily for 5 days. According to the invention, the cycle is preferably repeated as necessary to achieve suppression of disease symptoms. Further information about suitable dosages is provided in the Examples below.

VI. 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, a label and a 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 on, or associated with, the container indicates that the composition is used for treating the condition of choice. 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. In addition, the article of manufacture may comprise a package inserts with instruc-
Further details of the invention are illustrated by the following non-limiting Examples.

EXAMPLES

Preparation and Efficacy of HERCEPTIN® Anti-ErbB2 Antibody

Materials and Methods

Anti-ErbB2 monoclonal antibody The anti-ErbB2 IgG, 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 W089/06692. Briefly, NIH 3T3/HER2-3400 cells (expressing approximately 1x10^6 ErbB2 molecules/cell) produced as described in Hudeczak et al., Proc. Natl. Acad. Sci. (USA) 84:7159 (1987) were harvested with phosphate buffered saline (PBS) containing 25 mM EDTA and used to immunize BALB/c mice. The mice were given injections i.p. of 10^7 cells in 0.5 ml PBS on weeks 0, 2, 5 and 7. The mice with antisera that immunoprecipitated 32 P-labeled ErbB2 were given i.p. injections of a wheat germ agglutinin-Sepharose (WGA) purified ErbB2 membrane extract on weeks 9 and 13. This was followed by an i.v. injection of 0.1 mg/kg of murine 4D5 MAb, which has the same CDRs as the humanized antibody used for the treatment. Tumors are considered to over-express ErbB2 if at least 25% of tumor cells exhibit characteristic membrane staining for p_A5HER2.

Bidimensionally measurable disease (including lytic bone lesions) by radiographic means, physical examination, or photographs.

Measurable disease was defined as any mass reproducibly measurable in two perpendicular diameters by physical examination, X-ray (plain films), computerized tomography (CT), magnetic resonance imaging (MRI), ultrasound, or photographs.

Osteoblastic metastases, pleural effusions, or ascites were not considered to be measurable. Measurable lesions must be at least 1 cm in greatest dimension. Enumeration of evaluable sites of metastatic disease and number of lesions in an evaluable site (e.g. lung) had to be recorded on the appropriate Case Report Form (CRF). If a large number of pulmonary or hepatic lesions were present, the six largest lesions per site were followed.

The ability to understand and willingness to sign a written informed consent form

Women ≥ 18 years

Suitable candidates for receiving concomitant cytotoxic chemotherapy as evidenced by screening laboratory assessments of hematologic, renal, hepatic, and metabolic functions.

Exclusion Criteria

Patients with any of the following were excluded from study entry:

1. Prior cytotoxic chemotherapy for metastatic breast cancer
2. Patients may have received prior hormonal therapy (e.g. tamoxifen) for metastatic disease or cytotoxic therapy in the adjuvant setting.
3. Concomitant malignancy that has not been curatively treated
4. A performance status of ≤50% on the Karnofsky scale
5. Pregnant or nursing women; women of childbearing potential, unless using effective contraception as determined by the investigator
6. Bilateral breast cancer (either both primary tumors must have 2+ to 3+ HER2 overexpression, or the metastatic site must have 2+ to 3+ HER2 overexpression)
7. Use of investigational or unlicensed agents within 30 days prior to study entry
8. Clinically unstable or untreated metastases to the brain (e.g. requiring radiation therapy)
9. Based upon the foregoing criteria, 469 patients were chosen, and enrolled in the study. Half the patients (stratified by chemotherapy) were randomized to additionally receive the HERCEPTIN® anti-ErbB2 antibody (see below).

Administration and Dosage

On day 0, a 4 mg/kg dose of humanized anti-ErbB2 antibody (HERCEPTIN®, H) was administered...
intravenously, over a 90-minute period. Beginning on day 7, patients received weekly administration of 2 mg/kg antibody (i.v.) over a 90-minute period.

Chemotherapy

The patients received one of two chemotherapy regimens for a minimum of six cycles, provided their disease was not progressing: a) cyclophosphamide and doxorubicin or epirubicin (AC), if patients have not received anthracycline therapy in the adjuvant setting, or b) paclitaxel (TAXOL®), if patients have received any anthracycline therapy in the adjuvant setting. The initial dose of the HERCEPTIN® anti-ErbB2 antibody preceded the first cycle of either chemotherapy regimen by 24 hours. Subsequent doses of the antibody were given immediately before chemotherapy administration, if the initial dose of the antibody was well tolerated. If the first dose of the antibody was not well tolerated, subsequent infusions continued to precede chemotherapy administration by 24 hours. Patients were permitted to continue receiving chemotherapy beyond six cycles if, in the opinion of the treating physician, they were continuing to receive treatment benefit.

Cyclophosphamide (600 mg/m²) was given either by iv push over a minimum period of 3 minutes or by infusion over a maximum period of 2 hours.

Doxorubicin (60 mg/m²) or epirubicin (75 mg/m²) were given either by slow iv push over a minimum period of 3–5 minutes or by infusion over a maximum period of 2 hours, according to institutional protocol.

Paclitaxel (TAXOL®) was given at a dose of 175 mg/m² over 3 hours by intravenous administration. All patients receiving paclitaxel were premedicated with dexamethasone (or its equivalent) 20 mgx2, 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.

Response Criteria

Objective evidence of an increase of 25% or more in any measurable lesion. Progressive disease also includes those instances when new lesions have appeared. For bone lesions, progression is defined as a 25% increase in objective measurement by plain film, CT, MRI; symptomatic new lesions or new lesions which were not due to fracture; or requirement for palliative radiotherapy.

Complete Response

Disappearance of all radiographically and/or visually apparent tumor for a minimum of 4 weeks. Skin and chest wall complete responses had to be confirmed by biopsy.

Partial Response

A reduction of at least 50% in the sum of the products of the perpendicular diameters of all measurable lesions for a minimum period of 4 weeks. No new lesions may have appeared, nor may any lesions have progressed in size.

Minor Response

A reduction of 25% to 49% in the sum of the products of the perpendicular diameters of all measurable lesions. No new lesions may have appeared, nor may any lesions have progressed in size.

Stable Disease

No change of greater than 25% in the size of measurable lesions. No lesions may have appeared.

Time to disease progression (TTP) was calculated from the beginning of therapy to progression. Confidence limits for response rates were calculated using the exact method for a single proportion. (Fleiss, J.L., Statistical Methods for Rates and Proportions (ed.2), New York, N.Y., Wiley, 1981, pp 13–17).

Results

At a median follow-up of 10.5 months, assessments of time to disease progression (TTP in months) and response rates (RR) showed a significant augmentation of the chemotherapeutic effect by HERCEPTIN® anti-ErbB2 antibody, without increase in overall severe adverse events (AE):

<table>
<thead>
<tr>
<th>HERCEPTIN® Anti-ErbB2 Antibody Efficacy</th>
<th>Enrolled</th>
<th>TTP (months)</th>
<th>RR (%)</th>
<th>AE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRx</td>
<td>234</td>
<td>5.5</td>
<td>36.2</td>
<td>66</td>
</tr>
<tr>
<td>CRx + 14</td>
<td>235</td>
<td>8.6*</td>
<td>62.00**</td>
<td>69</td>
</tr>
<tr>
<td>AC</td>
<td>148</td>
<td>6.5</td>
<td>42.1</td>
<td>71</td>
</tr>
<tr>
<td>AC + H</td>
<td>146</td>
<td>9.0</td>
<td>64.9</td>
<td>68</td>
</tr>
<tr>
<td>T</td>
<td>89</td>
<td>4.2</td>
<td>25.0</td>
<td>59</td>
</tr>
<tr>
<td>T + H</td>
<td>89</td>
<td>7.1</td>
<td>57.3</td>
<td>70</td>
</tr>
</tbody>
</table>

* p < 0.001 by log-rank test; ** p < 0.01 by X² test; CRx: chemotherapy; AC: anthracycline/cyclophosphamide; H: HERCEPTIN® anti-ErbB2 antibody; T: TAXOL®

A syndrome of myocardial dysfunction similar to that observed with anthracyclines was reported more commonly with a combined treatment of AC+H (18% Grade III) than with AC alone (3%), T (0%), or T+H (2%).

These data indicate that the combination of anti-ErbB2 antibody treatment with chemotherapy markedly increases the clinical benefit, as assessed by response rates and the evaluation of disease progression. However, due to the increased cardiac side-effects of doxorubicin or epirubicin, the combined use of anthracyclines with anti-ErbB2 antibody therapy is contraindicated. The results, taking into account risk and benefit, favor treatment with HERCEPTIN® anti-ErbB2 antibody and paclitaxel (TAXOL) where a combined treatment regimen is desired.

Example 2

Pharmacokinetic and Pharmacodynamic Properties of Anti-ErbB2 Antibody (HERCEPTIN®)

HERCEPTIN® anti-ErbB2 antibody was administered by intravenous, infusion to human patients selected according to the criteria provided in Example 1. An initial dose of 4 mg/kg HERCEPTIN® anti-ErbB2 antibody was delivered by intravenous infusion, followed by subsequent i.v. infusions of 2 mg/kg HERCEPTIN® anti-ErbB2 antibody weekly for several weeks. Two hundred thirteen patients began this treatment regimen and serum drug concentration was obtained beyond 8 weeks for fewer than 90 patients as selective discontinuation of patients with rapidly progressing disease occurred. Of the 213 patients who began treatment, serum trough concentration data were available for 80 patients at Week 12, for 77 patients at Week 16, for 44 patients at Week 20, for 51 patients at Week 24, for 25 patients at Week 28, for 23 patients at Week 32, and for 37 patients at Week 36.

The HERCEPTIN® anti-ErbB2 Antibody Trough Serum Concentrations for Weeks 0–36

The HERCEPTIN® anti-ErbB2 antibody trough serum concentrations (µg/mL, mean±SE) from Week 2 through Week 36 are plotted in FIG. 3 (dark circles). The number of patients was fairly constant because data from patients discontinued from the program due to rapidly progressing disease were excluded from this analysis. Trough serum concentrations tended to increase through Week 12 and tended to plateau after that time.

HERCEPTIN® anti-ErbB2 Antibody Trough and Peak Serum Concentrations for Weeks 1–8

Some HERCEPTIN® anti-ErbB2 antibody serum concentration data were available for 212 of the original 213 patients. Trough and peak serum concentration data reflecting the first HERCEPTIN® anti-ErbB2 antibody infusion
were available for 195 of the 212 patients. For the seventh infusion, trough serum concentration data were available for 137/212 patients and peak serum concentration data were available for 114/212 patients. Table 2 presents a summary of statistics from trough and peak serum concentrations for the first 8 weeks of treatment. Peak samples were drawn shortly after the end of HERCEPTIN® anti-ErbB2 antibody administration; trough samples were drawn prior to the subsequent dose (i.e., 1 week later). Serum concentrations of HERCEPTIN® anti-ErbB2 antibody were determined as disclosed herein.

**TABLE 2**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Number</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak</td>
<td>1</td>
<td>195</td>
<td>100.3</td>
<td>35.2</td>
<td>80.7</td>
</tr>
<tr>
<td>Trough</td>
<td>2</td>
<td>190</td>
<td>74.3</td>
<td>31.3</td>
<td>20.8</td>
</tr>
<tr>
<td>Peak</td>
<td>3</td>
<td>167</td>
<td>67.0</td>
<td>29.8</td>
<td>40.2</td>
</tr>
<tr>
<td>Trough</td>
<td>4</td>
<td>158</td>
<td>50.0</td>
<td>25.7</td>
<td>20.8</td>
</tr>
<tr>
<td>Peak</td>
<td>5</td>
<td>138</td>
<td>42.1</td>
<td>26.9</td>
<td>22.2</td>
</tr>
<tr>
<td>Trough</td>
<td>6</td>
<td>128</td>
<td>38.6</td>
<td>21.0</td>
<td>2.8</td>
</tr>
<tr>
<td>Peak</td>
<td>7</td>
<td>117</td>
<td>35.2</td>
<td>22.8</td>
<td>20.8</td>
</tr>
<tr>
<td>Trough</td>
<td>8</td>
<td>107</td>
<td>28.2</td>
<td>20.8</td>
<td>10.3</td>
</tr>
</tbody>
</table>

The data in Table 2 suggest that there was an increase in trough serum concentration over time. Of the many patients studied, there were 18 patients for whom the trough concentrations did not exceed 20 μg/ml from Week 2 through Week 8. A HERCEPTIN® anti-ErbB2 antibody trough serum concentration of 20 μg/ml was nominally targeted for these studies based on prior pharmacologic studies in animals and exploratory analyses in clinical trials.

Patient response status was evaluated relative to serum concentration of HERCEPTIN® anti-ErbB2 antibody. For this purpose, mean serum concentration (an average of troughs and peaks) was calculated for various times and patient response status (where the patient response status was determined by an independent Response Evaluation Committee). The increase in serum concentration between Weeks 2 and 8 appeared to be greater in responders than in nonresponders, suggesting that there is a relationship between response status and HERCEPTIN® anti-ErbB2 antibody serum concentration. A statistical analysis (analysis of variance) of trough serum concentration values at Week 2 and an average of Weeks 7 and 8 in relation to response status indicated a highly significant relationship between response status and average trough of Weeks 7 and 8 (p<0.001). The results indicated that there was a significant difference between the trough serum concentration (average troughs of Weeks 7 and 8) in the responders and nonresponders: trough concentrations were 60±20 μg/ml in the responders versus 44±25 μg/ml in the nonresponders (mean±SD). HER2 overexpression level and type of metastatic sites were associated with significant differences in trough serum concentrations. At Week 2, patients with 2+ HER2 overexpression had significantly higher trough serum concentrations (p=0.001, mean=28.8 μg/ml, SD=10.4) compared with patients with 3+ HER2 overexpression (n=155, mean=24.1 μg/ml, SD=13.1). This difference in the average trough serum concentrations for Weeks 7 and 8 was no longer statistically significant. Further, at Week 2, patients with superficial disease had significantly higher trough serum concentrations (n=12, mean=34.1 μg/ml, SD=12.0) compared with patients with visceral disease (n=183, mean=24.4 μg/ml, SD=12.5). This difference in the average trough serum concentrations for Weeks 7 and 8 was significant. These data indicate that the rise in trough serum concentrations between Weeks 2 and 7/8 occurs for human patients with various disease profiles.

In a subsequent, similarly designed study, human breast cancer patients were treated with a loading dose of 8 mg/kg followed by maintenance doses of 4 mg/kg weekly. The results of this preliminary human study indicated that an 8mg/kg load:4 mg/kg weekly maintenance regimen was efficacious in reducing tumor volume in the patients.

The data disclosed in this Example indicate that front loading of antibody, such that a target serum concentration is reached more quickly, may be associated with improved outcomes.

Example 3

I.V. Bolus Delivery and Subcutaneous Infusion of HERCEPTIN® Anti-ErbB2 Antibody Effectively Decrease Tumor Volume in the Mouse

The efficacy of infusion or bolus delivery of humanized anti-ErbB2 antibody (HERCEPTIN®, see Example 1 for preparation), either by intravenous injection or subcutaneous injection, was examined. The purpose of the study was to ask whether subcutaneous delivery was feasible and whether the convenient subcutaneous bolus delivery was useful in treating metastatic breast cancer in animals inoculated with a cell line that overexpresses the HER2 gene. The results, detailed below, show that i.v. and s.c. infusion and bolus delivery are feasible treatment methodologies.

A study in a nude mouse xenograft model, which incorporates a human breast cancer cell line that naturally over-expresses the HER2 gene (BT-474MI, derived from BT-474 cells, ATCC Accession number HTB-20), comparing tumor volume as a function of i.v. bolus versus s.c. infusion was performed as follows. In the first study athymic nude nu nu 7–9 week old female mice were obtained from Taconic Inc. (Germantown, N.Y.). To initiate tumor development, each mouse was inoculated subcutaneously with 3x10^5 B174M 1 cells suspended in Matrigel™. When tumor nodules reached a volume of approximately 100 mm³, animals were randomized to 4 treatment groups. The groups were treated according to Table 3.

**TABLE 3**

<table>
<thead>
<tr>
<th>Group, Dose, Antibody</th>
<th>Target Serum Conc. (μg/ml)</th>
<th>Route of Administration</th>
<th>Loading Dose Maintenance (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Control, rhuMAb E25</td>
<td>20</td>
<td>IV LD and SC infusion</td>
<td>2.20</td>
</tr>
<tr>
<td>2-Low Dose SC rhuMAb HER2</td>
<td>1</td>
<td>IV LD and</td>
<td>0.313</td>
</tr>
<tr>
<td>3-High Dose SC rhuMAb HER2</td>
<td>20</td>
<td>IV LD and</td>
<td>6.25</td>
</tr>
<tr>
<td>4-IV Multi-Dose rhuMAb HER2</td>
<td>20</td>
<td>IV LD and MD</td>
<td>4.00</td>
</tr>
</tbody>
</table>

Animals were exposed to estrogen by subcutaneous sustained release estrogen pellet 9 days before the start of...
dosing to promote growth of grafted tumor cells. The animals were inoculated with the BT474M 1 cells 8 days before the beginning of treatment and tumors were allowed to grow. The animals were then treated with irrelevant antibody E25 (non-specific for HER2 receptor, but a member of the monoclonal IgG class) or test antibody HERCEPTIN® anti-ErbB2 antibody as indicated in Table 3. The dosage levels were selected to achieve target serum concentrations of HERCEPTIN®, either 1 μg/ml or 20 μg/ml, by subcutaneous pump infusion or by i.v. bolus delivery. The study groups were treated until day 35. The serum concentration of HERCEPTIN® anti-ErbB2 antibody was measured weekly (Oust prior to dosing for Group 4) using 3 mice/group/time point. The anti-ErbB2 antibody concentration was determined according to the method disclosed herein involving standard techniques. Tumor volumes were measured two days before dosing began and twice per week from day 0 to day 35 in the study for which data is tabulated below. Tumors were measured in three dimensions and volumes expressed in mm³. Efficacy was determined by a statistical comparison (ANOVA) of tumor volumes of test animals relative to untreated control animals. As shown in Table 4, below, treatment of the BT474M 1 tumor-bearing mice with HERCEPTIN® anti-ErbB2 antibody by the indicated dosage methods significantly inhibited the growth of the tumors. All HERCEPTIN®-treated groups showed similar inhibition of tumor growth relative to the control group. No dose-response was observed.

### TABLE 4

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Tumor Volume (mm³)</th>
<th>Tumor Volume (area under curve)</th>
<th>HERCEPT® Serum Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control s.c. infusion</td>
<td>764 ± 300</td>
<td>1565 ± 4700</td>
<td>4.16 ± 1.94</td>
</tr>
<tr>
<td>s.c. infusion (low dose)</td>
<td>90.5 ± 158</td>
<td>1610 ± 1250</td>
<td>2.11 ± 1.74</td>
</tr>
<tr>
<td>s.c. infusion (high dose)</td>
<td>31 ± 75.6</td>
<td>1440 ± 1140</td>
<td>22.1 ± 5.43</td>
</tr>
<tr>
<td>i.v. bolus dose*</td>
<td>49.7 ± 95.7</td>
<td>2150 ± 1480</td>
<td>21.7 ± 17.1**</td>
</tr>
</tbody>
</table>

s.c. = subcutaneous delivery; i.v. = intravenous.
*4.0 mg/kg Loading Dose and 2.0 mg/kg/week Maintenance Dose.
**at predose (trough serum concentration immediately prior to a maintenance dose).

The results tabulated above indicate that maintenance of a serum concentration of approximately 2 μg/ml was as effective as a concentration of 20 μg/ml in this study. The results indicated that dosing by subcutaneous infusion was as effective as intravenous bolus dosing and achieved similar trough serum concentrations. The results also indicate that the dose levels studied are at the top of the dose-response curve in this model and that subcutaneous dosing is effective in treating breast cancer tumors. Thus, subcutaneous administration of maintenance doses is feasible as part of a HERCEPTIN® anti-ErbB2 antibody treatment regimen.

### Example 4

I.V. Bolus and Subcutaneous Bolus Deliveries of HERCEPTIN® Anti-ErbB2 Antibody Effectively Decrease Tumor Volume in the Mouse

Subcutaneous bolus delivery is convenient and cost-effective for the patient and health care professionals. The results of the study disclosed in this example indicate that subcutaneous bolus delivery was as effective as intravenous bolus delivery in reducing breast cell tumor size in a mouse.

This study was set up as disclosed herein in Example 3 for the comparison of intravenous bolus and subcutaneous infusion delivery. A sustained release estrogen implant was inserted subcutaneously one day before tumor cell inoculation as described in Example 3. Six days after tumor cell inoculation, the initial tumor measurement was performed. Seven days after tumor cell inoculation, the first dose of control antibody or HERCEPTIN® anti-ErbB2 antibody was delivered. The animal groups, type of delivery, loading dose and maintenance doses are provided in Table 4. Animals were dosed once weekly for 4 weeks.

### TABLE 5

<table>
<thead>
<tr>
<th>Group</th>
<th>Route of Administration</th>
<th>Loading Dose (μg/kg)</th>
<th>Maintenance Dose (μg/kg/week)</th>
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</thead>
<tbody>
<tr>
<td>1-Control</td>
<td>IV</td>
<td>8</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>rhMAB E25</td>
<td>IV</td>
<td>2</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>3-rhumAB HER2</td>
<td>IV</td>
<td>4</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>4-rhumAB HER2</td>
<td>IV</td>
<td>8</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>5-rhumAB HER2</td>
<td>SC</td>
<td>4</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

IV = intravenous; SC = subcutaneous; n = number of animals per group.

The mice were treated according to the information in Table 4 and using the techniques disclosed in Example 3. The serum concentration of HERCEPTIN® anti-ErbB2 antibody was measured weekly before each weekly i.v. maintenance dose according to the procedure described herein and using standard techniques. The control E25 antibody serum concentration was determined according to standard immunoassay techniques. Table 6 shows the increase in HERCEPTIN® anti-ErbB2 antibody serum concentrations with time.

### TABLE 6

<table>
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<tr>
<th>Treatment Group (delivery, MD)</th>
<th>Day 0 Mean (SD)</th>
<th>Day 7 Mean (SD)</th>
<th>Day 14 Mean (SD)</th>
<th>Day 21 Mean (SD)</th>
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<tr>
<td>1-Control</td>
<td>0</td>
<td>25.9 (8.29)</td>
<td>34.6 (12.2)</td>
<td>36.5 (14.6)</td>
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<tr>
<td>2-rhumAB HER2</td>
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<td>4.96 (7.9)</td>
<td>8.55 (8.5)</td>
<td>8.05 (8.4)</td>
</tr>
<tr>
<td>3-rhumAB HER2</td>
<td>0</td>
<td>13.4 (14.4)</td>
<td>18.9 (13.6)</td>
<td>22.6 (15.9)</td>
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<tr>
<td>4-rhumAB HER2</td>
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<td>29.6 (7.35)</td>
<td>37.7 (10.2)</td>
<td>46.2 (10.7)</td>
</tr>
<tr>
<td>5-rhumAB HER2</td>
<td>0</td>
<td>12.5 (13.5)</td>
<td>16.9 (14.4)</td>
<td>17.6 (15.9)</td>
</tr>
</tbody>
</table>

n = 10 for time points Days 0, 7, and 14.
N = 9 for Day 21.

Table 7 shows the relative efficacy of intravenous bolus delivery and subcutaneous bolus delivery for Groups 1–5 having achieved the serum antibody concentrations presented in Table 6. For this study, efficacy was measured as a decrease in tumor volume. Tumor volume was measured twice weekly.

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Tumor growth rate calculated on Day 21-Day 31 Log(TM + 1). Area under the curve is the area beneath a plot of tumor volume versus time.

be less frequent and/or delivered by subcutaneous injection, sufficient to achieve an efficacious target serum concentration in approximately 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, and most preferably 1 week or less, including one day or less. According to the invention, this initial dosing is followed by dosing that maintains the target serum concentration by subsequent doses equal to or smaller than the initial dose or doses, consistent with an object of the invention of administering HERCEPTIN® anti-ErbB2 antibody by regimens providing greater front loading. The specific drug delivery regimens disclosed herein are representative of the invention and are not meant to be limiting.

In one trial, an initial dose of 6 mg/kg, 8 mg/kg, or 12 mg/kg of HERCEPTIN® anti-ErbB2 antibody is delivered to human patients by intravenous or subcutaneous injection. Initial doses (loading doses) are delivered by intravenous infusion or bolus injection or preferably subcutaneous bolus injection. Preferably a target trough serum concentration of HERCEPTIN® anti-ErbB2 antibody of approximately 10-20 μg/ml is achieved (averaged for all patients in the treatment group) and maintained by subsequent doses of anti-ErbB2 antibody that are equal to or smaller than the initial dose. In one method, a target trough serum concentration is achieved and maintained by once-per-week deliveries of 2 mg/kg HERCEPTIN® anti-ErbB2 antibody by intravenous or subcutaneous injection for at least eight weeks. Alternatively, for this or any dosage regimen disclosed herein, subcutaneous continuous infusion by subcutaneous pump is used to deliver subsequent maintenance doses.

In another method, an initial (front loading) dose of 8 mg/kg HERCEPTIN® anti-ErbB2 antibody is delivered by intravenous infusion (injection or bolus injection) or by subcutaneous bolus injection. This is followed by intravenous bolus injections, intravenous infusion, subcutaneous infusion, or subcutaneous bolus injection of 6 mg/kg at 3-week intervals to maintain a trough serum concentration of approximately 10-20 μg/ml, averaged for an entire treatment group.

In another method, an initial (front loading) dose of 12 mg/kg HERCEPTIN® anti-ErbB2 antibody is delivered by intravenous infusion (injection or bolus injection) or by subcutaneous bolus injection. This is followed by intravenous bolus injections, intravenous infusion, subcutaneous infusion, or subcutaneous bolus injection of 6 mg/kg at 3-week intervals to maintain a trough serum concentration of approximately 10-20 μg/ml.

In yet another method, an initial (front loading) dose of 8 mg/kg HERCEPTIN® anti-ErbB2 antibody is delivered by intravenous infusion (injection or bolus injection) or preferably by subcutaneous bolus injection or infusion. This is followed by administration of 8 mg/kg per week or 8 mg/kg per 2-3 weeks to maintain a trough serum concentration of HERCEPTIN® anti-ErbB2 antibody of approximately 10-20 μg/ml. Maintenance doses are delivered by intravenous infusion (injection or bolus injection), or preferably by subcutaneous infusion or bolus injection.

In another method, the front loading initial dose is a series of intravenous or subcutaneous injections, for example, one on each of days 1, 2, and 3 of at least 1 mg/kg for each injection (where the amount of anti-ErbB2 antibody delivered by the sum of initial injections is more than 4 mg/kg), followed by maintenance doses of 6 mg/kg once each 3 week interval to maintain a target trough serum concentration for approximately 10-20 μg/ml of HERCEPTIN® anti-ErbB2 antibody. The maintenance doses are delivered by intravenous infusion or bolus injection or by subcutaneous infusion or subcutaneous bolus injection.
In yet another method, the front loading is by intravenous infusion of at least 1 mg/kg, preferably 4 mg/kg on each of five consecutive days, followed by repeats of this cycle a sufficient number of times to achieve suppression of disease symptoms. Following the initial dose or doses, subsequent doses may be delivered by subcutaneous infusion or bolus injection if tolerated by the patient. Such subcutaneous delivery is convenient and cost-effective for the patient and administering health care professionals.

In still another method, HERCEPTIN® anti-ErbB2 antibody is delivered initially as at least 2 intravenous infusions per week for three weeks, followed by repeats of this cycle to maintain an efficacious trough serum concentration of HERCEPTIN® anti-ErbB2 antibody. The dose is at least 4 mg/kg of anti-ErbB2 antibody, preferably at least 5 mg/kg. The maintenance drug deliveries may be intravenous or subcutaneous.

Where the animal or patient tolerates the antibody during and after an initial dose, delivery of subsequent doses may be subcutaneous, thereby providing greater convenience and cost-effectiveness for the patient and health care professionals.

In animal studies, an initial dose of more than 4 mg/kg, preferably more than 5 mg/kg delivered by intravenous or subcutaneous injection, is followed by subcutaneous bolus injections of 2 mg/kg twice per week (separated by 3 days) to maintain a trough serum concentration of approximately 10–20 μg/ml. In addition, where the animal or patient is known to tolerate the antibody, an initial dose of HERCEPTIN® anti-ErbB2 antibody, followed by subcutaneous bolus injection followed by subcutaneous maintenance injections.

While target serum concentrations are disclosed herein for the purpose of comparing animal studies and human trials, target serum concentrations in clinical uses may differ. The disclosure provided herein guides the user in selecting a front loading drug delivery regimen that provides an efficacious target trough serum concentration.

The methods of the invention disclosed herein optionally include the delivery of HERCEPTIN® anti-ErbB2 antibody in combination with a chemotherapeutic agent (other than an anthracycline derivative) to achieve suppression of disease symptoms. The chemotherapeutic agent may be delivered with HERCEPTIN® anti-ErbB2 antibody or separately and according to a different dosing schedule. For example, subcutaneous delivery of HERCEPTIN® anti-ErbB2 antibody with TAXOL® is included in the invention. In addition, intravenous or subcutaneous injection of 8 mg/kg HERCEPTIN® anti-ErbB2 antibody, followed by intravenous or subcutaneous injection of 6 mg/kg HERCEPTIN® anti-ErbB2 antibody every 3 weeks is administered in combination with a chemotherapeutic agent, such as a taxoid (e.g. paclitaxel 175mg/m² every 3 weeks) or an anthracycline derivative (e.g. doxorubicin 60 mg/m² or epirubicin 75 mg/m² every 3 weeks). Optionally, where an anthracycline derivative is administered, a cardioprotectant (e.g. 600 mg/m² cyclophosphamide every 3 weeks) is also administered. In another combination therapy, anti-ErbB2 antibody is administered in a loading dose of more than 4 mg/kg, preferably more than 5 mg/kg, and more preferably at least 8 mg/kg. The loading dose is followed by maintenance doses of at least 2 mg/kg weekly, preferably 6 mg/kg every 3 weeks. The combination therapy includes administration of a taxoid during treatment with anti-ErbB2 antibody. According to one embodiment of the invention, the taxoid is paclitaxel and is administered at a dose of 70–100 mg/m² weekly. According to another embodiment of the invention, the taxoid is docetaxel and is administered at a dose of 30–70 mg/m² weekly.

Example 6

HERCEPTIN®-Administered Intravenously Every Three Weeks in Combination with Paclitaxel

Currently, the recommended dose of HERCEPTIN® is 2 mg/kg once weekly. Patients will be administered HERCEPTIN® every three weeks instead of weekly, along with paclitaxel (175 mg/m² every three weeks). Simulation of the proposed treatment regimen suggests that the trough serum concentrations will be 17 mcg/ml in the range (10–20mcg/ml) of the targeted trough serum concentrations from previous HERCEPTIN® IV clinical trials. After the first 12 patients the PK parameters will be assessed, if exposure is felt inadequate, then the dose will be increased to 8 mg/kg every three weeks for the remaining 12 patients.

Inclusion Criteria

1) Females ≥ 18 years of age
2) Histologically confirmed ErbB2 over-expressing metastatic breast cancer
3) Patients who have been newly diagnosed with metastatic disease
4) Have a Karnofsky performance status of ≥ 70%
5) Give written informed consent prior to any study specific screening procedures with the understanding that the patient has the right to withdraw from the study at any time, without prejudice.

Exclusion Criteria

1) Pregnant or lactating women
2) Women of childbearing potential unless (1) surgically sterile or (2) using adequate measures of contraception such as oral contraceptive, intra-uterine device or barrier method of contraception in conjunction with steroidal jelly.
3) Clinical or radiologic evidence of CNS metastases.
4) History of any significant cardiac disease
5) LVEF ≤ 50%
6) No prior taxane therapy in any treatment setting.
7) Any of the following abnormal baseline hematologic values:
   - Hb less than 9 g/dl
   - WBC less than 3.0x10⁹/l
   - Platelets less than 100x10⁹/l
   - Granulocytes less than 1.5x10⁹/l
8) Any of the following abnormal baseline liver function tests:
   - Serum bilirubin greater than 1.5x ULN (upper normal limit)
   - ALT and/or AST greater than 2.5x ULN (greater than 4.0x ULN if liver or bone metastasis)
   - Alkaline phosphatase greater than 2.5x ULN (greater than 4.0x ULN if liver or bone metastasis)
9) The following abnormal baseline renal function tests:
   - Serum creatinine greater than 1.5x ULN
10) History of other serious medical conditions that would preclude patient participation in an investigational study.

HERCEPTIN® Loading dose and schedule: 8 mg/kg for first dose. Maintenance dose and schedule: 6 mg/kg every 3 weeks.

Paclitaxel—175 mg/m² IV every 3 weeksx6 cycles as a 3-hour infusion. NOTE: On the first cycle of treatment,
It is believed that the above treatment regimen will be effective in treating metastatic breast cancer, despite the infrequency with which HERCEPTIN® is administered to the patient. While the particular aspects and embodiments of the invention as herein shown and disclosed in detail is fully capable of obtaining the objects and providing the advantages herein before stated, it is to be understood that it is merely illustrative of some of the presently preferred embodiments of the invention and that no limitations are intended to the details of methods and articles of manufacture shown other than as described in the appended claims. The disclosures of all citations in the specification are expressly incorporated herein by reference.

SEQUENCE LISTING

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Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Asp
50 55 60
Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile
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35 40 45
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50 55 60
Glu Gln Arg Phe Lys Gly Lys Ala Ser Leu Thr Val Asp Arg Ser
85 70 75
Ser Arg Ile Val Tyr Met Gln Leu Arg Ser Leu Thr Phe Glu Asp
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35 40 45
Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
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Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
50 55 60
Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser
85 70 75
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90
Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly Pro Ser Phe Tyr
95 100 105
What is claimed is:

1. A method for the treatment of a human patient diagnosed with cancer characterized by overexpression of ErbB2 receptor, comprising administering an effective amount of an anti-ErbB2 antibody to the human patient, the method comprising:

   - administering to the patient an initial dose of at least approximately 5 mg/kg of the anti-ErbB2 antibody; and
   - administering to the patient a plurality of subsequent doses of the antibody in an amount that is approximately the same or less than the initial dose, wherein
the subsequent doses are separated in time from each other by at least three weeks.

2. The method of claim 1, wherein the initial dose is at least approximately 6 mg/kg.

3. The method of claim 2, wherein the initial dose is at least approximately 8 mg/kg.

4. The method of claim 3, wherein the initial dose is at least approximately 12 mg/kg.

5. The method of claim 1, wherein the subsequent doses are separated in time from each other by at least three weeks.

6. The method of claim 1, wherein the initial dose is administered by intravenous injection, and wherein at least one subsequent dose is administered by subcutaneous injection.

7. The method of claim 1, wherein the initial dose is administered by intravenous injection, wherein at least two subsequent doses are administered, and wherein each subsequent dose is administered by a method selected from the group consisting of intravenous injection and subcutaneous injection.

8. The method of claim 1, wherein the initial dose and at least one subsequent dose are administered by subcutaneous injection.

9. The method of claim 1, wherein the initial dose is selected from the group consisting of approximately 6 mg/kg, 8 mg/kg, or 12 mg/kg, wherein the plurality of subsequent doses are at least approximately 2 mg/kg.

10. The method of claim 9, wherein the plurality of subsequent doses are separated in time from each other by at least three weeks.

11. The method of claim 10, wherein the initial dose is approximately 8 mg/kg, and wherein at least one subsequent dose is approximately 6 mg/kg.

12. The method of claim 10, wherein the initial dose is approximately 12 mg/kg, and wherein at least one subsequent dose is approximately 6 mg/kg.

13. The method of claim 9, wherein the initial dose is approximately 8 mg/kg, and wherein at least one subsequent dose is approximately 8 mg/kg.

14. The method of claim 9, wherein the initial dose is approximately 8 mg/kg, wherein at least one subsequent dose is 8 mg/kg, and wherein administration of the initial dose and subsequent doses are separated in time by at least 2 weeks.

15. The method of claim 14, wherein the initial dose and subsequent doses are separated in time by at least 3 weeks.

16. A method for the treatment of a human patient diagnosed with cancer characterized by overexpression of ErbB2 receptor, comprising administering an effective amount of an anti-ErbB2 antibody to the human patient, the method comprising:

administering to the patient an initial dose of the antibody, wherein the initial dose is a plurality of doses, wherein each of the plurality of initial doses is at least approximately 1 mg/kg and is administered on at least 3 consecutive days, and administering to the patient at least 1 subsequent dose of the antibody, wherein at least one subsequent dose is at least approximately 6 mg/kg, and wherein administration of the last initial dose and the first subsequent and additional subsequent doses are separated in time by at least 3 weeks.

17. The method of claim 1, wherein said cancer is selected from the group consisting of breast cancer, leukemia, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

18. The method of claim 17, wherein said cancer is breast cancer.

19. The method of claim 18, wherein said cancer is metastatic breast carcinoma.

20. The method of claim 1, wherein said antibody binds to the extracellular domain of the ErbB2 receptor.

21. The method of claim 20, wherein said antibody binds to epitope 4D5 within the ErbB2 extracellular domain sequence.

22. The method of claim 21, wherein said antibody is a humanized 4D5 anti-ErbB2 antibody.

23. The method of claim 1, wherein efficacy is measured by determining the time to disease progression or the response rate.

24. A method for the treatment of cancer in a human patient comprising administering to the patient a first dose of an anti-ErbB2 antibody followed by two or more subsequent doses of the antibody, wherein the subsequent doses are separated in time from each other by at least two weeks.

25. The method of claim 24, wherein the first dose and a first subsequent dose are separated from each other in time by at least about three weeks.

26. The method of claim 24, wherein the first dose and subsequent doses are each from about 2 mg/kg to about 16 mg/kg.

27. The method of claim 26, wherein the first dose and subsequent doses are each from about 4 mg/kg to about 12 mg/kg.

28. The method of claim 27, wherein the first dose and subsequent doses are each from about 6 mg/kg to about 12 mg/kg.

29. The method of claim 24, wherein from about two to about ten subsequent doses of the antibody are administered to the patient.

30. The method of claim 24, wherein the subsequent doses are separated in time from each other by at least about three weeks.

31. The method of claim 24, wherein the two or more subsequent doses are each from about 2 mg/kg to about 16 mg/kg.

32. The method of claim 24, wherein the two or more subsequent doses are each from about 4 mg/kg to about 12 mg/kg.

33. The method of claim 24, wherein the two or more subsequent doses are each from about 6 mg/kg to about 12 mg/kg.

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