No. 2019-2156

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

GENENTECH, INC.,

Plaintiff-Appellant,

CITY OF HOPE,

Plaintiff,

v.

AMGEN INC.,

Defendant-Appellee.

On Appeal from the United States District Court
for the District of Delaware, No. 1:18-cv-00924-CFC, Judge Colm F. Connolly

NON-CONFIDENTIAL BRIEF FOR PLAINTIFF-APPELLANT GENENTECH, INC.

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CERTIFICATE OF INTEREST

Counsel for Plaintiff-Appellant Genentech, Inc. certifies the following:

1. The full name of every party or *amicus* represented by me is:

Genentech, Inc.

2. The names of the real party in interest represented by me are:

Not applicable.

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curiae represented by me are:

Genentech, Inc. is a wholly-owned subsidiary of Roche Holdings Inc. Roche Holdings Inc.'s ultimate parent, Roche Holdings Ltd, is a publicly held Swiss corporation traded on the Swiss Stock Exchange. Upon information and belief, more than 10% of Roche Holdings Ltd's voting shares are held either directly or indirectly by Novartis AG, a publicly held Swiss corporation.

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in this court (and who have not or will not enter an appearance in this case) are:

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5. The title and number of any case known to counsel to be pending in this or any other court or agency that will directly affect or be directly affected by this court's decision in the pending appeal:

Genentech, Inc. v. Amgen Inc., No. 18-cv-924-CFC (D. Del.)

Dated: July 26, 2019

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STATEMENT OF RELATED CASES

This is an interlocutory appeal following the denial of a preliminary injunction in *Genentech, Inc. v. Amgen Inc.*, No. 18-cv-924-CFC (D. Del.). That case remains pending before the district court. Otherwise, counsel is aware of no case in this or any other court that would affect or be affected by the outcome of this appeal.

JURISDICTIONAL STATEMENT

This is an appeal from the denial of a preliminary injunction in a patent-infringement action. The district court had jurisdiction under 28 U.S.C. §§ 1331 and 1338(a). The district court denied Genentech's motion for a preliminary injunction on July 18, 2019. Appx11. Genentech filed its notice of appeal the following day, on July 19, 2019. Appx4885-4904. This Court has jurisdiction under 28 U.S.C. § 1292(a)(1) and (c)(1).

INTRODUCTION

Genentech holds three patents on the most prescribed dosing regimen for its blockbuster drug Herceptin®. As of last Thursday, July 18, 2019—when the district court issued the order being appealed here—Herceptin was the only commercially available therapy using these patented inventions. Had the preliminary injunction been granted, it would have remained such until [redacted]

[redacted], when the first licensed Herceptin biosimilar would have launched as a result of a settlement.

The district court refused a preliminary injunction. Notably, the district court did not address whether Genentech was likely to succeed on the merits, although the evidence overwhelmingly favors Genentech: Amgen conceded that its biosimilar version of Herceptin, Kanjinti, [redacted]. And although Amgen challenges the patents' validity (via attorney argument unsupported by any expert testimony), it makes essentially the same arguments that were rejected in final written decisions in recent IPR proceedings under a less demanding standard of proof. Nor did the district court address the balance of hardships. Instead, the district court premised its denial of a preliminary injunction entirely on the theory that Genentech would not be irreparably harmed by Amgen's launch. It made two legal errors in doing so.

First, the district court incorrectly held that Genentech could not show irreparable harm because of its timing in seeking injunctive relief. But Amgen only made the decision to launch at risk on [redacted], and Genentech moved for a preliminary injunction [redacted] days later. Importantly, Genentech's motion was filed before Amgen's intended launch, which was to occur beginning on [redacted]. Although a patentee's claim to irreparable harm may fairly be questioned where it has been suffering the alleged irreparable harm for an extended period of

time without seeking relief, such an inference cannot be drawn when the patentee moves for a preliminary injunction ***before*** the harm has even begun to occur.

The district court's opinion suggests that Genentech should have sought an injunction upon receipt of Amgen's notice of commercial marketing pursuant to 42 U.S.C. § 262(*l*)(8) on May 15, 2018, but Kanjinti was not even approved by the FDA until over a year later, on June 13, 2019, and [redacted].

Nor would it have made sense for Genentech to move for a preliminary injunction when Amgen received FDA approval on June 13, 2019. Even thereafter, Amgen continued to represent that it had not decided whether to launch at risk, and it insisted that disputes about a potential launch "***may not be ripe***" depending on Amgen's ultimate launch decision and timing. Appx1277(31:4-11) (emphasis added). Genentech filed emergency motions for a temporary restraining order and a preliminary injunction in the district court just [redacted] days after Amgen made its launch decision. Had Genentech filed materially earlier under the circumstances, its motion would have been premature. The district court's insistence that Genentech must not have faced irreparable harm because it did not seek a preliminary injunction before Amgen received FDA approval and before it made a launch decision was legal error and an abuse of discretion.

Second, the district court erroneously held that Amgen's July 2019 launch would not irreparably harm Genentech because Genentech had settled litigation with other biosimilar manufacturers by licensing them to enter the market in the future, even though those agreements allowed Genentech to maintain its market exclusivity until then. The court applied a categorical rule that ***any*** license—even one that only licensed a party to enter the market in the future, where the licensor negotiated [redacted]—demonstrates the absence of irreparable harm by ***present*** infringement in a market where the patentee is the only party practicing the invention. That bright-line rule makes no sense and violates *eBay* and this Court's precedents. If anything, Genentech's decision to negotiate for [redacted] shows an inability to place a monetary value on Genentech's market exclusivity. The district court's conclusion that Genentech forfeited its right to obtain an injunction would mean, as a practical matter, that innovators cannot settle pending cases without losing their right to exclude other entrants in any subsequent litigation. This too is a legal error requiring reversal.

Given these errors in the district court's analysis, coupled with Genentech's strong showing on the merits, Genentech is entitled to an injunction that bars Amgen from launching its biosimilar product at least pending trial, which is scheduled for December 9, 2019. The district court's denial of such relief was an

abuse of discretion and should be reversed or, in the alternative, remanded with instructions to consider the availability of a preliminary injunction without regard to the timing of the filing or the fact that Genentech settled with other entrants. Should the Court grant Genentech's pending motion under Fed. R. App. P. 8 and subsequently remand for the district court for further consideration, Genentech respectfully requests that this Court order that the injunction pending appeal continue through the district court's determination on remand. *See Nat. Res. Def. Council Inc. v. Ross*, 2019 WL 2173792, at *3 (Fed. Cir. May 20, 2019) (nonprecedential).

STATEMENT OF ISSUE ON APPEAL

Whether the district court committed legal errors and abused its discretion in denying Genentech's motion for a preliminary injunction.

STATEMENT OF THE CASE

A. Genentech's Development Of Herceptin And The Dosing Patents

Roughly 25-30% of women diagnosed with breast cancer each year have "HER2-positive" cancer, which causes them to overexpress human epidermal growth factor 2 ("HER2"). Appx1492(¶19). HER2-positive breast cancer is particularly aggressive: In the 1990s, before there were any targeted treatments, it was "associated with poor prognosis," with cancer progressing and spreading

quickly and patients with advanced disease having a life expectancy of only 18 months. Appx1492(¶20).

Starting in the 1980s, Genentech developed Herceptin, which fundamentally changed the treatment of HER2-positive breast cancer. Herceptin's active ingredient is "trastuzumab," a monoclonal antibody that targets and arrests the growth of HER2-positive cancers. Appx1492-1493(¶21). Following FDA approval in 1998, Herceptin was hailed as a revolution—demonstrating for the first time that solid tumors could be treated with a targeted therapy. Appx1493(¶22). Since then, Herceptin has extended and, in early breast cancer, saved the lives of hundreds of thousands of patients. Appx1467-1468(¶12); Appx1493-1494(¶¶23-24). Indeed, due to Genentech's research, HER2-positive breast cancer has gone from having the worst prognosis of any breast cancer to one of the best. Appx1466-1467(¶¶6-9). Herceptin is now the standard of care for HER2-positive breast cancer. Appx1467-1468(¶¶9-12).

Genentech's work did not stop with Herceptin's approval in 1998. Genentech continued to invest billions of dollars and countless hours of research in the intervening two decades to improve therapeutic options for HER-2 positive patients. Appx1467(¶7). This investment of resources was high risk and included investing in clinical trials to extend the use of Herceptin from advanced (i.e., metastatic) breast cancer to early breast cancer patients, who could be given the

drug in a curative setting following surgery (referred to as "adjuvant" therapy). Appx1467(¶8).

Of particular relevance here, Genentech researchers successfully developed new dosing regimens that make Herceptin more convenient for early breast cancer patients by extending the intervals between clinical visits from one week to three weeks. Appx1494(¶25). Specifically, Genentech researchers discovered how to administer the drug in ways that allow for longer intervals between doses while still maintaining efficacy, including a method of administering a larger initial dose of 8 mg/kg trastuzumab followed by subsequent doses of 6 mg/kg every three weeks. Appx1494(¶26). This 8/6 three-weekly dosing regimen is claimed in U.S. Patent Nos. 6,627,196 ("the '196 patent"), 7,371,379 ("the '379 patent"), and 10,160,811 ("the '811 patent") (collectively, "the dosing patents"), and recited in each of the claims asserted in this case. Appx1494-1496(¶¶27-30).[1]

The Herceptin label includes the patented dosing regimen as one of two options for adjuvant treatment, instructing administration of an "[i]nitial dose of 8 mg/kg over 90 minutes IV infusion, then 6 mg/kg over 30-90 minutes IV

[1] The asserted claims include claims 11 and 22 of the '196 patent, claims 11 and 21 of the '379 patent, and claims 6 and 7 of the '811 patent. *See* Appx49('196 patent claims 11 & 22); Appx87-88('379 patent claims 11 & 21); Appx139('811 patent claims 6 & 7).

infusion every three weeks for 52 weeks." Appx1680-1685. This 8/6 three-weekly dosing is also the ***only*** dosing regimen for gastric cancer. *Id.*

As between the two options for adjuvant treatment, the 8/6 three-weekly dosing regimen is most often prescribed. Appx1496-1497(¶32); Appx2325; Appx2335. According to Amgen's own documents, the claimed dosing regimen constitutes up to [redacted] of the market. Appx2499; Appx1696-1697(198:21-199:1); Appx1699-1702(205:16-208:24); Appx1827.

Biosimilar applicants recognized the significance of Genentech's patents on these inventions and challenged two of the dosing patents—the '196 and '379 patents—through *inter partes* review. The Patent Trial and Appeal Board (PTAB) rejected these challenges in October 2018, and the appeals to this Court have been dismissed. Final Written Decision, *Hospira, Inc. v. Genentech, Inc.*, IPR2017-00804 (P.T.A.B. Oct. 3, 2018) ('196 patent); Final Written Decision, *Celltrion, Inc. v. Genentech, Inc.*, IPR2017-01139 (P.T.A.B. Oct. 3, 2018) ('196 patent); Final Written Decision, *Hospira, Inc. v. Genentech, Inc.*, IPR2017-00805 (P.T.A.B. Oct. 3, 2018) ('379 patent); Final Written Decision, *Celltrion, Inc. v. Genentech, Inc.*, IPR2017-01140 (P.T.A.B. Oct. 3, 2018) ('379 patent). In each of its final written decisions, the PTAB found that the petitioners did not show, by a preponderance of the evidence, that the challenged claims were invalid. The PTAB explained that the "Petitioners have not pointed to any prior art reference

discussing the feasibility or viability of a tri-weekly antibody dosing regimen," and that the "evidence shows that the prior art did not contain sufficient data from which the skilled artisan could reliably predict the plasma concentration for trastuzumab over a three-week dosing interval using a one-compartment model." Final Written Decision at 23, 25, *Hospira, Inc. v. Genentech, Inc.*, IPR2017-00804. The third dosing patent, the '811, issued after the IPRs concluded and is narrower than the other dosing patents; its notice of allowance references the IPR final written decisions. Appx1231; Appx89-139.

In order to settle various litigations and IPRs brought by other biosimilar applicants, Genentech has entered into settlement agreements authorizing them to enter the market on agreed-to future dates, with the earliest entry date allowed under these settlements being [redacted]. [redacted] in connection with those agreements, because Genentech recognized that the value of its hard-earned market exclusivity, protected in part by the dosing patents, could not be quantified or adequately compensated by a [redacted]. As of last Thursday, July 18, when the district court refused to enter a TRO or a preliminary injunction, Genentech's Herceptin was the only product on the market utilizing Genentech's dosing patents.

B. Amgen's Biosimilar Drug Kanjinti

Amgen developed a biosimilar version of Herceptin called Kanjinti. Long before the FDA approved the product, Amgen sent Genentech a Notice of Commercial Marketing under 42 U.S.C. § 262(*l*)(8) on May 15, 2018. At that time, [redacted]. After the PTAB upheld the '196 and '379 patents' validity in the IPRs in October 2018, Amgen asked the FDA [redacted]. Appx1830; Appx1832. But Amgen subsequently determined that a [redacted] Appx1837; Appx1694-1695(179:5-14, 179:24-180:4); Appx1705(27:19-28:11); Appx1676-1678(234:5-24; 239:10-240:10). Even thereafter, Amgen continued to consider [redacted]. Appx4074 ("[redacted]"). And throughout this time, FDA approval was not guaranteed; indeed, Amgen's initial application was not approved, and, following receipt of a complete response letter from the FDA in May 2018, Amgen was

required to resubmit its application to the FDA in December 2018. Appx6; Appx3770(¶3).

The FDA ultimately approved Amgen's Kanjinti biosimilar on June 13, 2019 with a label materially identical to Herceptin's. Appx1583; *see also* Appx1687 (Amgen press release stating that FDA "has approved KANJINTI™ (trastuzumab-anns) for all approved indications of the reference product, Herceptin (trastuzumab)"). Given that both labels recite Genentech's patented 8/6 three-weekly dosing regimens, Amgen [redacted] the dosing patents. Appx3759.

C. Amgen's Representations Regarding The Timing Of Its Launch Of Kanjinti

When Amgen gave Genentech its Notice of Commercial Marketing of Kanjinti in May 2018, Amgen was precluded from launching not only by the statutory 180-day waiting period, *see* 42 U.S.C. § 262(*l*)(8), but also because it had not received FDA approval. Indeed, Amgen did not receive FDA approval until over a year later, on June 13, 2019. Unsurprisingly, given the complexities of preparing for a launch, Amgen began those preparations well before FDA approval, and targeted being ready to launch by July 2019. But Amgen's actual launch timing remained undetermined: in a deposition on June 26-27, 2019, Molly Benson—[redacted]

[redacted]

Appx4838(353:12-19) (objection omitted).

[redacted]

Appx4835(98:1-16).

Amgen's other witnesses also testified that [redacted]. *See, e.g.*, Appx4841 (May 24, 2019 Hall Dep. Tr. at 72:9-14) ("Q. [redacted]." (objection omitted; emphasis added)); Appx4844 (April 30, 2019 Yant Dep. Tr. at 62:19-62:23) ("Q. [redacted]

[redacted]

Amgen's counsel likewise represented to Genentech and the district court that no decision had been made. Even after receiving FDA approval, Amgen explained that, notwithstanding its launch planning, an actual launch could still be as much as a year away, putting it well after the scheduled December 2019 trial date. Appx1277 (June 18, 2019 Hearing Tr. at 30:3-8) ("[I]f we were to launch in ***two months versus six months versus a year***, the company would still need to make the preparation to be in a position to launch[.]" (emphasis added)). And in view of these uncertainties, Amgen argued that disputes relating to a potential launch "***may not be ripe***" because "***the circumstances from Genentech's standpoint and from the market standpoint may be very different*** in those different scenarios." Appx1277(31:4-11; 30:3-8) (emphasis added). Indeed, Amgen's counsel flatly told the district court that that it had not determined whether it would ***actually*** launch Kanjinti at risk:

> Part of the problem is ***we have not made that ultimate decision yet*** because we have not launched yet. We have not launched yet. That's what I'm saying. ***Those decisions are ongoing.***

Appx1289(78:22-25) (emphasis added). Amgen then reiterated in a brief filed the following week that its launch decision was "***something that has not occurred***." Appx1299 (emphasis added).

Amgen did not make the decision to launch until [redacted] And, when Amgen made that decision, it decided to launch not within two months, or six months, or a year, but just [redacted] later, on [redacted]. Appx3770(¶5). Amgen did not tell Genentech that it had decided to launch within [redacted]; Genentech learned about Amgen's forthcoming launch through its own market intelligence and immediately brought a motion for a preliminary injunction in the district court on July 10, five days before Amgen's intended launch date.

D. District Court Proceedings

Genentech filed this patent infringement action against Amgen on June 21, 2018, shortly after receiving Amgen's original Notice of Commercial Marketing. Fact discovery closed on June 10, 2019; the parties are now in the midst of expert discovery, with trial currently scheduled for December 9, 2019. Appx1001-1004; Appx1011-1023; Appx1233-1235.

During the week of July 8, 2019, Genentech for the first time received concrete market intelligence that Amgen was planning an imminent launch of Kanjinti. On July 10, 2019—[redacted] days after Amgen had decided to launch, and [redacted] days before the intended [redacted] launch—Genentech filed emergency motions

for a temporary restraining order and preliminary injunction in the district court. Appx1324-1325; Appx1332-1333. Genentech addressed each of the four factors for injunctive relief, presenting the expert declaration of Dr. Susan Tannenbaum on infringement and the fact declaration of Christy Oliger and expert declaration of Dr. Anupam Jena outlining the irreparable harm that Genentech would suffer if Amgen launched its infringing Kanjinti product. Specifically, Genentech explained that Kanjinti infringed the dosing patents, and that its launch would cause, among other things, erosion to the price of Herceptin and a significant loss of market share, consequences that Amgen itself has argued elsewhere could not be remedied by money damages. Appx1931-1935. The district court entered a standstill order that same day. Appx3738-3739.

Amgen filed an opposition on July 15, but did not provide any supporting expert declarations, even though the deadline for expert reports was only 10 days later. Appx3746, Appx1234. Amgen [redacted]. Appx3759. With respect to invalidity, Amgen largely rehashed the same arguments that had been presented in the IPRs, addressed to art that was cumulative of the art that the PTAB had already considered. In support of those arguments, Amgen provided only attorney argument without any supporting expert testimony. Nor did Amgen dispute that price erosion and loss of market share suffice to demonstrate irreparable harm—likely because Amgen itself has relied upon price erosion and

loss of market share to establish irreparable harm when it is the patent owner. Appx1931-1935. Genentech filed its reply the following day, providing the supplemental declaration of Ms. Oliger and the expert declaration of Dr. George Grass addressing the patents' validity. Appx4720-4789.

On July 18, 2019, the district court entered an order denying Genentech's motions. Appx11. The district court ruled that Genentech had not shown that Amgen's launch would cause it irreparable harm, for two reasons. First, the court asserted that Genentech's failure to move for an injunction earlier negated a finding of irreparable harm, even though Amgen had not yet launched Kanjinti and despite the fact that Amgen consistently represented—even after the FDA's approval on June 13, 2019—that it had not decided whether to launch at risk. Appx6-7. Second, the court found that Genentech's agreement that other companies could enter the market "in [redacted]" meant that Genentech had an adequate remedy at law, because any damages accruing before trial "should be quantifiable." Appx8-9. The court cited no evidence for that conclusion, nor did it address Genentech's evidence that price erosion and loss of market share were not compensable by money damages—a showing Amgen had not disputed.

The district court did not consider Genentech's likelihood of success on the merits or the balance of hardships. It considered the public interest only in a footnote, which stated a concern that, because the Kanjinti label (like the Herceptin

label) contains "two recited methods … that are free of any allegations of infringement," an injunction would "'depriv[e] the public of access to a large number of non-infringing features.'" Appx9-10 n.7 (quoting *Apple Inc. v. Samsung Elecs. Co.*, 735 F.3d 1352, 1372-1373 (Fed. Cir. 2013)). The court did not address the fact that Genentech's Herceptin product, which is already in the market and can supply all public need, is likewise indicated for those "two recited methods," such that Kanjinti does not fill any market need currently unmet by Herceptin.

Very shortly after receiving the district court's order, Genentech advised Amgen that it planned to appeal to this Court and to ask the district court and, if necessary, this Court for an interim injunction pending appeal. Appx4938-1439(25:7-26:15); Appx4945(32:2-10). After meeting and conferring with Amgen the following morning (Friday, July 19), Genentech filed its notice of appeal and a motion for injunction pending appeal under Federal Rule of Civil Procedure 62(d); Genentech alternatively sought a short 14-day injunction to enable Genentech to ask this Court to enter an injunction pending appeal under Federal Rule of Appellate Procedure 8. Appx4885-4904; ECF No. 9.

After holding a telephonic hearing, the district court denied Genentech's Rule 62(d) motion at 4:45 p.m. that same day (Friday, July 19). Appx4964-4966(51:4-53:22). Genentech filed its motion in this Court for an injunction

pending appeal under Federal Rule of Appellate Procedure 8 at 6:30 p.m. that day; Genentech also asked for the Court to expedite briefing on the motion and on the appeal. ECF No. 9. On Monday morning, July 22, 2019, this Court accepted Genentech's motion for filing and ordered Amgen to respond to it by Monday, July 29, 2019. ECF No. 10.

Genentech is filing this opening brief on the merits on an expedited basis, within one week of its notice of appeal.

SUMMARY OF THE ARGUMENT

The district court's determination that Genentech will suffer no irreparable harm was based on two legal errors that are inconsistent with precedent governing preliminary injunctive relief and that, unless reversed, will make it extremely difficult for innovator companies to settle biosimilar litigation under the framework of the Biologics Price Competition and Innovation Act (BPCIA).

First, the district court was wrong to conclude that Genentech would not suffer irreparable harm from Amgen's launch because it filed its preliminary injunction request after Amgen had decided to launch an infringing product, but before the launch itself. Appx5-6. The timing of an injunction motion is logically relevant to irreparable harm to the extent that a party's delay demonstrates that it is willing to tolerate that harm by taking no action to prevent it. *See Nutrition 21 v. United States*, 930 F.2d 867, 872 (Fed. Cir. 1991). But this Court has never held

that a party that seeks an injunction before the harm to be enjoined even begins to occur has somehow forfeited the ability to show irreparable harm. Genentech moved for relief [redacted] days after Amgen made its launch decision, and [redacted] days before the intended launch date of [redacted] The circumstances here amply explain why Genentech did not seek an injunction earlier: Amgen consistently represented to Genentech and the district court that—despite targeting a launch "readiness" date of July 2019—it had not even decided whether to launch at risk, up through and following the date of Kanjinti's approval. *See, e.g.*, Appx1277(30:3-8); Appx1289(78:22-25). Genentech filed its motion as soon as it learned that Amgen would launch at risk; that Amgen's launch was merely days away has nothing to do with Genentech's irreparable harm and everything to do with the timing of Amgen's decision-making.

To the extent the district court viewed the BPCIA as requiring Genentech to act sooner, that was error. A notice under the relevant statutory section may be a necessary condition to moving for a preliminary injunction, but it does not require a reference-product sponsor like Genentech to seek a preliminary injunction immediately upon receipt of a notice of commercial marketing when, under the circumstances, such a motion would be premature. This case illustrates why a contrary rule is unworkable: between issuing its (*l*)(8) notice in May 2018 and FDA approval in June 2019, Amgen was forced to resubmit its application to the

FDA and [redacted]. Appx3770(¶3); Appx1830; Appx1832; Appx1837-1839. It would make no sense to require Genentech to seek a preliminary injunction on the basis of patents that had been [redacted]; had Genentech moved for a preliminary injunction upon service of the (*l*)(8) notice, it would have had to withdraw it when Amgen [redacted].

Second, the district court's reliance on Genentech's license agreements to find a lack of irreparable harm, without considering the significant differences between Genentech's settlement agreements allowing other parties to enter the market in the future and Amgen's present infringement, amounts to a categorical rule that such settlements negate irreparable harm. *Apple Inc. v. Samsung Elecs. Co.*, 735 F.3d 1352, 1370 (Fed. Cir. 2013) ("*Apple III*"). Such a rule is contrary to the Supreme Court's decision in *eBay, Inc. v. MercExchange, L.L.C.*, 547 U.S. 388, 393 (2006), which held that licensing of the patented invention does not preclude injunctive relief. Genentech's settlement agreements do not show that monetary damages could compensate for infringement. On the contrary, Genentech's decision to license other market entrants in the future without [redacted]

[redacted], rather than accepting an earlier entry date coupled with a [redacted] only confirms that it cannot put a [redacted] on its lawful exclusivity. *See AstraZeneca LP v. Apotex, Inc.*, 633 F.3d 1042, 1061-62 (Fed. Cir. 2010) (affirming preliminary injunction against unlicensed market entrant even though a competitor was licensed to enter in the future, because harm after the licensed entry would be impossible to calculate). Finally, unlike the companies with which Genentech settled, Amgen is uniquely positioned to do damage in the Herceptin market because of its relationships with oncology clinics, its experience with payers in the oncology market, and its established track record as a successful biologic manufacturer. Appx1477-1478(¶¶53-54); Appx1396-1397(¶59).

The district court's conclusion that under these circumstances Genentech's harms from Amgen's launch would be quantifiable finds no support in the record. There was simply no evidence supporting that proposition—the only evidence was Genentech's evidence showing the exact opposite. *E.g.*, Appx1399-1402(¶¶65-72); *see Sanofi-Synthelabo v. Apotex, Inc.*, 470 F.3d 1368, 1382 (Fed. Cir. 2006).

Once the district court's errors are corrected, the remaining record evidence establishes Genentech's entitlement to a preliminary injunction. First, as Genentech's expert and fact declarants testified—without any rebuttal from Amgen—Kanjinti's launch will erode the price of Genentech's Herceptin product and cause Genentech to lose market share. It will also cause collateral harm to

other Genentech products, harm from lost research opportunities, and harm to Genentech's reputation. This Court routinely finds that such harm is irreparable. *E.g.*, *Abbott Labs. v. Sandoz, Inc.*, 544 F.3d 1341, 1361-1362 (Fed. Cir. 2008). Second, the record evidence shows that Genentech is likely to succeed on the merits because Amgen did not dispute [redacted] and merely rehashed, without evidence, invalidity arguments previously rejected by the PTAB under a lower standard of proof. Third, the balance of hardships likewise weighs in Genentech's favor because any harm Amgen might suffer from an interim injunction pending trial is entirely self-inflicted due to Amgen's decision to launch at risk while knowing of Genentech's infringement claims. *Celsis In Vitro, Inc. v. CellzDirect, Inc.*, 664 F.3d 922, 931 (Fed. Cir. 2012). Finally, the public interest favors enforcing Genentech's patent rights, especially where Amgen does not contest [redacted] or that Genentech is capable of supplying the market.

The district court's denial of a preliminary injunction should be reversed, or at the very least vacated and remanded.

STANDARD OF REVIEW

In determining whether to grant a preliminary injunction, courts consider four factors: "(1) the likelihood of the patentee's success on the merits; (2) irreparable harm if the injunction is not granted; (3) the balance of hardships between the parties; and (4) the public interest." *Tinnus Enters., LLC v.*

Telebrands Corp., 846 F.3d 1190, 1202 (Fed. Cir. 2017); *accord Transcontinental Gas Pipe Line Co. v. Permanent Easements*, 907 F.3d 725, 732 (3d Cir. 2018).

This Court generally reviews preliminary injunction decisions under the law of the regional circuit because "'[t]he grant, denial, or modification of a preliminary injunction … is not unique to patent law.'" *Tinnus Enters.*, 846 F.3d at 1202-1203 (alterations in original) (quoting *Trebo Mfg., Inc. v. Firefly Equip., LLC*, 748 F.3d 1159, 1163 (Fed. Cir. 2014)). "'However, the Federal Circuit has itself built a body of precedent applying the general preliminary injunction considerations to a large number of factually variant patent cases, and gives dominant effect to Federal Circuit precedent insofar as it reflects considerations specific to patent issues.'" *Id.* at 1203 (quoting *Murata Mach. USA v. Daifuku Co.*, 830 F.3d 1357, 1363 (Fed. Cir. 2016)).

Both this Court and the Third Circuit review the grant or denial of a preliminary injunction for an abuse of discretion, which occurs when "the court made a clear error of judgment in weighing relevant factors or exercised its discretion based upon an error of law or clearly erroneous factual findings." *Tinnus Enters.*, 846 F.3d at 1203 (quoting *Abbott Labs. v. Andrx Pharm., Inc.*, 452 F.3d 1331, 1335 (Fed. Cir. 2006)); *see Reilly v. City of Harrisburg*, 858 F.3d 173, 176 (3d Cir. 2017) ("'When reviewing a district court's [denial] of a preliminary injunction, we review the court's findings of fact for clear error, its conclusions of

law de novo, and the ultimate decision … for an abuse of discretion.'" (alterations in original) (quoting *Bimbo Bakeries USA, Inc. v. Botticella*, 613 F.3d 102, 109 (3d Cir. 2010))).

ARGUMENT

I. THE DISTRICT COURT'S DETERMINATION OF NO IRREPARABLE HARM RESTED ON LEGAL ERRORS

A. The District Court Adopted An Erroneous Legal Standard To Conclude That Genentech Could Not Establish Irreparable Harm Because Of The Timing Of Its Motion

The district court's conclusion that Genentech could not show irreparable harm because of when it filed its motion reflects a misunderstanding of this Court's precedent on the proper timing of a preliminary injunction request and would require plaintiffs like Genentech to file unnecessary and premature motions in order to preserve their rights to exclude. When a party is suffering harm, waiting to file for a preliminary injunction may belie a later claim that that harm was truly irreparable. But that is not the situation here. Far from sitting idly by while Amgen captured its market, Genentech moved promptly, indeed with great speed, when it found out that Amgen had decided to launch. Amgen itself acknowledged that disputes might not be ripe prior to that point. There is no requirement for a patentee like Genentech to file a motion to enjoin sales that the infringer itself insists it may not make, and the district court abused its discretion in holding otherwise.

1. The district court legally erred in inferring that Genentech will not suffer irreparable harm because it waited to seek preliminary injunctive relief until Amgen affirmatively decided to launch

Amgen repeatedly represented, including in open court in late June 2019, that it had not decided whether to launch at risk, much less when it would do so. Genentech took Amgen at its word. *See supra* pp. 11-14. Once Genentech learned that Amgen had decided to launch, it moved for a preliminary injunction immediately. Genentech filed its preliminary injunction motion only [redacted] days after Amgen made its decision to launch and [redacted] days before Amgen's intended launch date. At the time Genentech filed its motion, Genentech had not yet suffered any harm from Kanjinti and would not have suffered any harm had its motions been granted.

Notwithstanding this record, the district court found that Genentech could not establish irreparable harm because of Genentech's supposed "undue delay" in seeking an injunction. Appx7. Although a patentee's claim to irreparable harm may be undermined where it has long suffered the alleged irreparable harm without complaint, such an inference cannot be drawn when the patentee moves before the harm has even begun. *See Nutrition 21*, 930 F.2d at 872 ("Finally, that Nutrition 21 delayed for a substantial period of time before seeking a preliminary injunction at least suggests that the *status quo* does not irreparably damage Nutrition 21."). This Court has never found undue delay where the patentee filed for preliminary

injunctive relief before the irreparable harm even began, simply because there was a ***possibility*** that irreparable harm might later take place.

Rather, this Court has found that undue delay defeats an irreparable-harm showing when a patentee who is actually suffering the harm of infringement does not seek injunctive relief for a lengthy period. For example, in *Apple, Inc. v. Samsung Electronics Co.*, 678 F.3d 1314, 1325-1326 (Fed. Cir. 2012), this Court affirmed the conclusion that a patentee's decision to wait to seek an injunction until one year after the launch of the most recent generation of infringing products weighed against irreparable harm. *Id.* at 1325-1326; *Apple, Inc. v. Samsung Elecs., Co.*, 2011 WL 7036077, at *22 (N.D. Cal. Dec. 2, 2011). Other cases considering a patentee's delay in moving for injunctive relief likewise involved lengthy periods when the supposedly harmful infringement ***was actually happening***. *See High Tech Med. Instrumentation, Inc. v. New Image Indus., Inc.*, 49 F.3d 1551, 1557 (Fed. Cir. 1995) (seventeen-month delay in seeking preliminary injunction after issuance of reexamination certificate weighed against a finding of irreparable harm when infringing product had been on the market for two years); *T.J. Smith & Nephew Ltd. v. Consolidated Med. Equip., Inc.*, 821 F.2d 646, 648 (Fed. Cir. 1987) (fifteen-month delay in seeking preliminary injunction after reissuance of patent weighs against finding irreparable harm where infringing product had been on the

market for three years) (affirming *T.J. Smith & Nephew Ltd. v. Consolidated Med. Equip., Inc.*, 645 F. Supp. 206 (N.D.N.Y. 1986)).

The cases the district court relied on actually support Genentech's position that a patentee who moves for an injunction before the irreparably harmful conduct occurs may show irreparable harm from imminent infringement—indeed, the successful patentees in those cases waited far longer than Genentech. In *Pfizer, Inc. v. Teva Pharmaceuticals, USA, Inc.*, 429 F.3d 1364 (Fed. Cir. 2005), this Court affirmed the district court's finding that the plaintiff had ***not*** unduly delayed in filing suit ***two months after the launch*** of the infringer's generic product and nearly two years after receiving notice of the defendant's paragraph IV certification. *Id.* at 1371, 1382. The Court in *Polymer Technologies, Inc. v. Bridwell*, 103 F.3d 970 (Fed. Cir. 1996), determined that filing suit ***four months*** after the commencement of infringing activity was a "short lapse of time" and did not negate irreparable harm. *Id.* at 976.[2]

[2] The cases Amgen cited in its opposition to Genentech's motion for a preliminary injunction are even farther afield. *Immunomedics, Inc. v. Venvio Select Advisor LLC*, 2017 WL 822800 (D. Del. Mar. 2, 2017), did not involve patent infringement, but rather held that the plaintiff's delay in seeking to enjoin a shareholder vote from replacing the board weighed against a finding of irreparable harm where it had known about the alleged violations of securities laws on that board's watch for six months and had already delayed the annual meeting twice. *Id.* at *3. *BMEF San Diego, L.L.C. v. Gray East Village San Diego L.L.C.*, 2014 WL 4923722 (Del. Ch. Sept. 30, 2014), involved a demand to expedite a trial in a dispute that was "largely about cash." *Id.* at *1-2. In *Graceway Pharms. LLC v. Perrigo Co.*, 722 F. Supp. 2d 566 (D.N.J. 2010), the court initially faulted the

Indeed, preliminary injunctions are routinely allowed after FDA approval and even after launch. *See, e.g.*, *Indivior Inc. v. Dr. Reddy's Labs. S.A.*, 2018 WL 3496643, at *4, *11-14 (D.N.J. July 20, 2018) (finding irreparable harm where plaintiff filed its preliminary injunction and TRO motions "on an emergent basis" "[u]pon learning of DRL's plans to launch the ANDA product 'at risk'"), *rev'd on other grounds*, 752 F. App'x 1024 (Fed. Cir. 2018) (nonprecedential); *Integra Lifesciences Corp. v. Hyperbranch Med. Tech., Inc.*, 2016 WL 4770244, at *8-9 (D. Del. Aug. 12, 2016) (recommending that the delay analysis should focus on the time the irreparable harm was to begin because "if a patentee is arguing that an accused infringer should be enjoined because a particular kind of infringing act is causing it irreparable harm, then the patentee would not seem to be properly motivated to seek an injunction until the infringer actually started to (or was about to) commit that particular infringing act") (collecting cases); *Ranbaxy Labs. Ltd. v. Abbott Labs.*, 2005 WL 3050608, at *29 (N.D. Ill. Nov. 10, 2005) (no undue delay in seeking preliminary injunction a year after FDA approval in ANDA litigation despite being "on notice" of generic infringers for two years, because patent holder

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plaintiffs for failing to inform the defendant of its intention to file suit upon the issuance of its patent, but changed its mind on a fuller record because "both parties could have acted more diligently." *Id.* at 569-570. It ultimately found no prejudice to the defendant in part because "Graceway brought suit (although not its TRO motion) prior to Nycomed's launch." *Id.* at 570. None of these cases stands for the proposition that a plaintiff acts too late even though the defendant has not yet begun to take the irreparably harmful action.

brought suit before defendant began marketing its product), *aff'd in part sub nom. Abbott Labs. v. Andrx Pharms., Inc.*, 473 F.3d 1196 (Fed. Cir. 2007).

In view of this precedent, the district court was wrong to determine that the timing of Genentech's motion defeated Genentech's showing of irreparable harm. It is not as though Genentech tolerated any harm and then later sought to call it irreparable; Genentech acted before the harm to be enjoined even began. Indeed, the record here is unequivocal: Genentech filed its motion for a preliminary injunction less than four weeks after Kanjinti was approved, within days of learning (not from Amgen, but through market intelligence) that Amgen was planning to launch; and [redacted] days before Amgen's intended launch date. Nothing in that conduct suggests that the harm Genentech would suffer from a launch was anything short of irreparable. It merely demonstrates that, because a preliminary injunction is an "extraordinary remedy," Genentech concluded it was inappropriate to burden the district court with a request for a preliminary injunction unless it was clearly necessary.

The consequence of the district court's rule reveals its unsoundness. Were the district court correct, patent owners would be obliged to move for preliminary injunctions when the fact of a launch, its timing, the exact nature of the product, and the commercial implications are still unknown. It is far from clear that an

injunction request would even be ripe in such a situation; it certainly is not ***required***.

The district court observed that Genentech had stated "as recently as May 16, 2019[,] that it was not seeking a preliminary injunction." Appx7 n.6. In fact, Genentech said, "We're not ***presently*** seeking injunctive relief" (Appx1246(26:3-4)), and it made that statement before the FDA had even approved Kanjinti. *See supra* pp. 11-14 (discussing statements made in June hearings and depositions). Genentech's truthful statement that it was not seeking a preliminary injunction in May when it was premature to do so should not foreclose it from filing a prompt motion when Amgen changed the game by making a launch decision, nor is it any evidence of a lack of irreparable harm once Amgen decided to launch.[3]

Any prejudice to Amgen from the timing of Genentech's motion is entirely a function of its own decision to make a decision to launch a [redacted] later. The district

[3] Amgen's decision to wait until [redacted] to make a launch decision, and then to decide to launch just a [redacted] later, is in stark contrast to what happened in a case involving another defendant's biosimilar version of Herceptin last year. The district court observed that the parties in that other case were able to "avoid hurried motion practice" (Appx7 n.6), but that was because that defendant, unlike Amgen, made a decision to launch months in advance of the actual launch date and informed the court and Genentech of that decision, which allowed the parties and the court to address the disputed issues in an orderly manner. Appx4948-4950(35:25-37:24). In the end, Genentech never filed a motion for preliminary injunction against that other biosimilar defendant because the parties settled the litigation.

court committed legal error and abused its discretion in holding that it instead reflected a lack of irreparable harm on Genentech's part.

2. Nothing in the BPCIA's text or purpose required Genentech to seek a preliminary injunction before Amgen decided that it was going to launch its infringing product

The district court also erred in stating that Genentech's actions were "contrary to the spirit and purpose" of the Biologics Price Competition and Innovation Act of 2009, Pub. L. No. 111-148, §§ 7001 *et seq.*, 124 Stat. 804 (codified in relevant part at, 42 U.S.C. § 262(*l*)(8)). Appx7. The district court seemed to suggest that the BPCIA required Genentech to move for a preliminary injunction when it received Amgen's notice of commercial marketing pursuant to 42 U.S.C. § 262(*l*)(8)(A) in May 2018. *Id.* To the extent the district court took such a view, it was legally erroneous.

The biosimilar applicant's notice of commercial marketing pursuant to § 262(*l*)(8)(A) will generally be a necessary condition to bringing a motion for a preliminary injunction. Indeed, the biosimilar product cannot come to market for at least 180 days after providing notice under § 262(*l*)(8)(A), and a motion for a preliminary injunction thus would not be appropriate until a biosimilar applicant provides such notice. But nothing in § 262(*l*)(8) requires a reference-product sponsor like Genentech to seek a preliminary injunction immediately upon receipt of a notice of commercial marketing when the circumstances do not warrant it.

The purpose of § 262(*l*)(8) is to make additional relief available[4]—not to require the reference-product sponsor to seek relief before it may even be ripe to do so.

The facts of this case illustrate why it makes no sense to require reference-product sponsors to seek preliminary injunctive relief upon receipt of an applicant's § 262(*l*)(8) notice of commercial marketing. Amgen issued that notice on May 15, 2018. But Kanjinti was not approved until 13 months later, on June 13, 2019. During that time, Amgen resubmitted its entire application due to its receipt of a complete response letter from FDA and [redacted]. Appx3770(¶3); Appx1675-1678(232:9-24, 234:5-24, 239:10-240:10); Appx1694-1695(179:5-14, 179:24-180:4); Appx1698(202:3-19); Appx1830; Appx1832; Appx1837-1839. Even after [redacted]. Appx4074.

[4] For example, the reference-product sponsor may seek a preliminary injunction or declaratory relief for additional patents after receiving a notice of commercial marketing. *See* 42 U.S.C. § 262(*l*)(8)(B) (preliminary injunction); *id.* § 262(*l*)(9)(A) (declaratory judgment).

Had Genentech moved for a preliminary injunction on the dosing patents when Amgen filed its § 262(*l*)(8) notice in May 2018 (or even 180 days after that), Amgen would no doubt have argued the motion was premature because the label was subject to change and its launch uncertain. As this Court has explained, "[i]t is not enough to show a 'possibility' of harm, as 'a possibility of irreparable harm is inconsistent with [the] characterization of injunctive relief as an extraordinary remedy that may only be awarded upon a clear showing that plaintiff is entitled to such relief.'" *IGT v. Aristocrat Techs., Inc.*, 646 F. App'x 1015, 1018 (Fed. Cir. 2014) (nonprecedential) (quoting *Winter v. Nat. Res. Def. Council, Inc.*, 555 U.S. 7, 22 (2008)). Instead, "[a] movant seeking a preliminary injunction must show that 'irreparable injury is *likely* in the absence of an injunction.'" *Id.* (quoting *Winter*, 555 U.S. at 22); *see also Cordis Corp. v. Medtronic, Inc.*, 780 F.2d 991, 996 (Fed. Cir. 1985) ("A preliminary injunction will not issue simply to prevent a mere possibility of injury, even where prospective injury is great."). A blanket rule requiring reference-product sponsors to file, and the courts to address, motions for preliminary injunction during a period when changes in the biosimilar product would potentially moot any need for such relief would waste court and party resources, and is contrary to the requirement of an imminent, irreparable injury necessary to obtain a preliminary injunction.

The district court quoted this Court's statements in *Amgen Inc. v. Apotex Inc.*, 827 F.3d 1052 (Fed. Cir. 2016), to suggest that the BPCIA requires filing a motion for preliminary injunction upon receiving the biosimilar applicant's notice of commercial marketing. Appx7. But that case was based on this Court's understanding at the time that a notice of commercial marketing could not be provided prior to FDA approval. 827 F.3d at 1062. The Supreme Court's subsequent decision in *Sandoz Inc. v. Amgen Inc.*, 137 S. Ct. 1664, 1677-1678 (2017), alters that analysis by holding that a notice of commercial marketing may be provided prior to FDA approval such that the applicant's actual commercial marketing is not imminent at the time that it provides notice under § 262(*l*)(8)(A).

Accordingly, nothing in the letter of the BPCIA or its "spirit and purpose" (Appx7) required Genentech to move for a preliminary injunction before it did: as soon as it knew that Amgen had decided to launch.

B. The District Court Improperly Created And Applied A Categorical Rule That Licenses To Third Parties For Future Entry Dates Demonstrated A Lack Of Irreparable Harm From Present Infringement

The district court further erred in holding that Genentech's settlement agreements allowing other defendants to enter the market in the future negated any showing of irreparable harm by Amgen's launch. The district court concluded that the fact that Genentech "granted [redacted] licenses" for the dosing patents in which it "approved competitors entering the market in a [redacted]"

somehow meant that Genentech had an adequate remedy at law for Amgen's market entry ***now***. Appx8-9. That was legal error and, unless reversed, would create a rule that would seriously impede settlements of biosimilar litigation.

1. The district court erred in adopting a categorical rule that licensing of *future* activity negates irreparable harm from *present* infringement

The district court adopted a categorical rule that settling a case against another entrant—even where the settlement is expressly for [redacted]—makes it impossible to establish irreparable harm and obtain injunctive relief, even where the settlement only permits entry at a future date. The district court's analysis is contrary to the Supreme Court's explicit rejection of the proposition that a patentee's willingness to license its patents suffices by itself to demonstrate a lack of irreparable harm. *eBay*, 547 U.S. at 393 (2006) ("To the extent that the District Court adopted such a categorical rule, then, its analysis cannot be squared with the principles of equity adopted by Congress."). It is also contrary to *Apple III*, where this Court reversed the denial of a preliminary injunction because "the district court's focus on Apple's past licensing practices, without exploring any relevant differences from the current situation, hints at a categorical rule that Apple's willingness to license its patents precludes the issuance of an injunction." 735 F.3d at 1370. That is exactly the same error that the district court made here, and it compels the same result.

This Court has explained that "[a] plaintiff's past willingness to license is not sufficient per se to establish lack of irreparable harm if a new infringer were licensed," and that "[a]dding a new competitor to the market may create an irreparable harm that the prior licenses did not." *Acumed LLC v. Stryker Corp.*, 551 F.3d 1323, 1328-29 (Fed. Cir. 2008). That is why, when considering whether infringement causes irreparable harm, "[t]he identity of the past licensees, the experience in the market since the licenses were granted, and the identity of the new infringer" are all factors for the court to consider, in addition to the bare fact of the grant of a license. *Id.* By failing to consider those factors—all of which demonstrate the sharp difference between Genentech's negotiated future licenses and the present license to Amgen that the district court forced onto Genentech here—the district court committed legal error. *Apple III*, 735 F.3d at 1370.

Had the district court made the proper comparison, the conclusion is inescapable that Genentech's settlement with other applicants who agreed not to enter the market before [redacted] in no way shows that Amgen's entry now would not cause irreparable harm. Genentech has not licensed ***any entry*** for the period between now and [redacted]. The litigation settlements show, if anything, that Genentech cannot put a [redacted] on exclusivity.

Genentech is not aware of any case in which a settlement allowing entry in the future has been found to defeat irreparable harm in the present. In facts, courts

have found the exact opposite. *See AstraZeneca*, 633 F.3d at 1061-63 (affirming preliminary injunction against unlicensed market entrant even though a competitor was licensed to enter in the future, because harm after the licensed entry would be impossible to calculate); *Abbott Labs. v. Sandoz, Inc.*, 500 F. Supp. 2d 807, 843 (N.D. Ill. 2007) (settlement agreements allowing two specific generic drugmakers to enter the market the following year did not mean that patent holder gave up its right to exclude generics in the present), *aff'd*, 544 F.3d 1341 (Fed. Cir. 2008); *cf. Nichia Corp. v. Everlight Ams., Inc.*, 855 F.3d 1328, 1343-1344 (Fed. Cir. 2017) (no irreparable harm where past "licenses ***changed the market by making available*** 'multiple low-priced non-infringing alternatives'" (emphasis added)).

Certainly none of the cases cited by the district court supports its proposition that licenses for future entry foreclose finding irreparable harm from an earlier entry. In *Polymer Technologies*, 103 F.3d at 974, there were no licenses at issue; that case merely cited the second case the district court relied upon, *High Tech Medical Instrumentation, Inc. v. New Image Industries, Inc.*, 49 F.3d 1551, 1557 (Fed. Cir. 1995), for the proposition that a party's licensing history can be a factor. *High Tech*, in turn, considered the patentee's "apparent willingness to grant a [royalty-bearing] license under its patent" not to a third party, but to the ***defendant itself***—a factor that unsurprisingly weighed against injunctive relief and that is undisputedly not present here. *Id.* *High Tech* is further distinguishable because the

patentee was a non-practicing entity—"the lack of commercial activity by the patentee" was a "significant factor" in the analysis. *Id.* at 1556. The cases Amgen cited in its opposition to Genentech's motion for a preliminary injunction are likewise inapposite. *See Cordis Corp. v. Boston Sci. Corp.*, 2003 WL 22843072, at *2 (D. Del. Nov. 21, 2003) (plaintiff had licensed major competitors who had competing (though non-infringing) products on the market), *aff'd*, 99 F. App'x 928 (Fed. Cir. 2004) (nonprecedential); *Cordance Corp. v. Amazon.com, Inc.*, 2010 WL 3155505, at *4 (D. Del. July 23, 2010) (plaintiff had licensed its patent to a nonprofit seeking to make an open-access platform available to anyone).

As Genentech has never agreed to license any competitor to enter the market before [redacted], the settlement agreements here in no way suggest that Genentech would not be irreparably harmed by Amgen's entry now. Indeed, as is discussed below, the harm from Amgen's unlicensed entry will continue past the licensed entry of other competitors.

2. The district court erred in treating Genentech's settlement agreements as establishing that money damages are an adequate remedy for Amgen's infringement

Genentech's willingness to allow other defendants to enter the market after [redacted], does not suggest that money damages from Amgen adequately compensate for Amgen's infringement in July 2019. License agreements may be considered as a factor in the irreparable-harm analysis where

they show that the patentee is less concerned with maintaining its exclusivity and is willing to trade it for cash. *See Acumed*, 551 F.3d at 1328 ("While the fact that a patentee has previously chosen to license the patent may indicate that a reasonable royalty does compensate for an infringement, that is but one factor for the district court to consider."). But the logic behind that rule is inapplicable here for several reasons.

First, none of the licenses at issue here provide a [redacted] for the right to practice Genentech's dosing patents, much less a [redacted] from Amgen's infringement. Genentech bargained for [redacted]. The fact that Genentech did not put a [redacted] on a license is further evidence that Amgen's unlicensed entry will cause irreparable harm and cannot be adequately remedied by money. *See, e.g.*, *High Tech*, 49 F.3d at 1557 ("[T]he evidence shows that HTMI offered a license to New Image, so it is clear that HTMI is willing to forgo its patent rights ***for compensation***" (emphasis added)); *Illinois Tool Works, Inc. v. Grip-Pak, Inc.*, 906 F.2d 679, 683 (Fed. Cir. 1990) ("ITW also argues that its grant of a license to Owens-Illinois, Inc. ***at a 4% royalty*** should not evidence an absence of irreparable harm, but does not tell us, nor can we discern, how any 'lost sales' attributable to Grip-Pak would be any less compensable in dollars than are the 'lost sales' attributable to Owens–Illinois, Inc." (emphasis added)).

Second, Genentech entered into those agreements to settle patent disputes. That Genentech was willing to trade some future exclusivity for certainty does not speak to whether money damages could adequately compensate for Amgen's infringement now. *See Trading Techs. Int'l, Inc. v. eSpeed, Inc.*, 2008 WL 4531371, at *4 (N.D. Ill. May 22, 2008) (finding, in the context of a permanent injunction, that the inadequate-remedy-at-law factor favored plaintiff where its past licenses "were negotiated in exchange for the parties' agreement to settle, rather than litigate"), *aff'd*, 595 F.3d 1340 (Fed. Cir. 2010).

Third, compared to the companies with which Genentech has settled, Amgen is an especially formidable competitive threat. *See Apple III*, 735 F.3d at 1370 ("the district court erred by failing to consider" the differences between Samsung and the other entities Apple had licensed). Here, Amgen is in a unique position to negotiate in the oncology market due to its multiple long-selling oncology products—including Neupogen and Neulasta—which have given it access to oncology clinics and experience with payers related to oncology products. Appx1477-1478(¶¶53-54); Appx1396-1397(¶59). Amgen has an established track record as a successful biologic manufacturer, and a reputation that will establish its credibility with oncology group purchasing organizations as a reliable, high-quality supplier of a biosimilar, which is not straightforward to manufacture. Appx1477-1478(¶54); Appx1396-1397(¶59). Furthermore, Kanjinti

is the only trastuzumab biosimilar to have transition data (i.e., data about switching from Herceptin to Kanjinti) as part of its clinical trial, a fact Amgen plans to advertise. Appx1477-1478(¶54); Appx1690; Appx2492-2493; Appx2496; Appx2574. For all these reasons, Amgen has a competitive advantage relative to other potential biosimilar entrants, enhancing the price-erosion and lost-market-share harms described below—which cannot be remedied by money damages. *Id.* Genentech therefore has a particular interest in keeping ***Amgen*** off the market, as the harms from Amgen's infringement will be especially acute.

3. The district court erred in concluding, without evidentiary support, that the harm to Genentech from Amgen's launch would be quantifiable

The district court further relied on the fact of the licenses to find that "any potential damages for sales in the next four months should be quantifiable." Appx9. The court cited no evidence for that proposition; in fact, the evidence is to the contrary, as Amgen itself has acknowledged when it has been the innovator seeking injunctive relief.

There is no evidence supporting the court's speculation that such harm "should be" quantifiable. Appx9. As discussed above, the settlement agreements the district court referred to do not contain [redacted] and so do not place a monetary value on Genentech's patent rights. The district court cited no other evidence of quantifiability, and it did not explain how such

damages would be calculated.[5] Amgen asserted this point in the district court with nothing more than a single, unsupported sentence of attorney argument. Appx3756. There was no basis to accept that conclusory argument in the face of undisputed evidence of irreparable harm presented by Genentech in the form of, inter alia, lost market share and price erosion. *See infra* pp. 44-51.

Genentech, on the other hand, introduced evidence showing that money damages for sales of Herceptin would be difficult to quantify in the months between now and trial. Genentech's responses to Amgen's entry will be multi-faceted and complex, and the specific effects of Amgen's activity—particularly those of price erosion and lost market share—will be difficult to unravel from other market conditions. Appx1399-1402(¶¶65-72); *see Sanofi-Synthelabo*, 470 F.3d at 1382 ("complex pricing scheme" for prescription drugs means additional entrants have potential to irreversibly erode prices in unpredictable ways). Indeed, Amgen itself has acknowledged these harms when it was the innovator seeking to exclude other market entrants. *See* Appx1922-1923; Appx1932-1934; Appx1939-1940.

Moreover, to the extent the district court assumed that the only harm that matters is harm to Genentech prior to the entry of other biosimilars, it was wrong.

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[5] The district court's citation to *King Pharmaceuticals, Inc. v. Sandoz, Inc.*, 2010 WL 1957640 (D.N.J. May 27, 2010), does not substitute for a determination that the damages ***in this case*** would be quantifiable. There, the court faulted the movant's expert's testimony for being conclusory. *Id.* at *5. Here, the district court did not even consider Genentech's evidence of irreparable harm.

Genentech presented detailed evidence, supported by expert testimony, that the irreparable harm from Amgen's entry would continue even after other biosimilars came onto the market, because it would then become more difficult to untangle the harms caused by Amgen's actions from those other entrants. Appx1401-1402(¶¶70-72); Appx1411-1413(¶¶98-100). Thus, far from ending a period of irreparable harm, the [redacted] date marks the day when the harm due to Amgen's unlicensed activity becomes even harder to quantify and even harder to compensate with money damages. *See AstraZeneca*, 633 F.3d at 1062 (affirming preliminary injunction because "to reliably calculate the economic harm" the patentee would suffer after the future licensed entry of another competitor, "the court would need data reflecting a market including only" the patentee and the licensed entrant, but the infringing entry of the defendant would prevent that from occurring, rendering those damages "complete speculation"). The district court's opinion ignored this evidence entirely.

The district court's unsupported finding that Genentech's harm was quantifiable was therefore clearly erroneous and based on its legal error regarding the relevance of the license agreements, and its denial of Genentech's preliminary injunction on this ground was an abuse of discretion.

4. Unless reversed, the district court's decision will seriously impede future settlement of biosimilars litigation

The district court's decision to hold these settlement agreements against Genentech would make litigation settlements in the pharmaceutical area much more difficult. Unless reversed, the district court's rule bars a patent owner from obtaining injunctive relief where it has licensed other parties, irrespective of when those parties are licensed to enter the market, who the parties are, and whether the licenses reflect a willingness of the patent holder to trade exclusivity for money. Such a rule would mean that a patentee could never settle litigation by permitting licensed entry, even at a future date, without giving up the opportunity to seek to enjoin immediate entry by anyone else who chooses to launch at risk.

C. Genentech Will Suffer Irreparable Harm Due To Amgen's Infringement

The district court did not even address Genentech's copious evidence of irreparable harm. But once the district court's errors are corrected, the only reasonable conclusion from the evidence presented is that Genentech will suffer irreparable harm from Amgen's launch. Indeed, Amgen did not dispute that Genentech will suffer price erosion and lost market share.

1. Genentech will suffer irreparable harm

By launching at risk, Amgen will cause incalculable harm to Genentech. Amgen will be the only Herceptin biosimilar on the market; absent an injunction,

Amgen's infringement will cause Genentech to suffer price erosion, lost market share, harm to other products, and damage to Genentech's reputation. *See, e.g.*, *Abbott Labs.*, 544 F.3d at 1361-62 (price erosion, loss or market position, loss of goodwill, and loss of research and development are examples of irreparable harm). Indeed, Amgen is well aware of these types of injury, many of which it has contended will irreparably harm its own innovative products in other litigation. *See* Appx1922 ("Price erosion alone is sufficient to establish irreparable harm."); Appx1933-1934 (conceding that an offer of discounts or rebates by a biosimilar maker in the oncology market "will irreparably harm" the reference-product sponsor by causing price erosion); Appx1932 ("Courts have repeatedly held that the steep loss of market share and revenue … caused by the introduction of a generic drug constitute irreparable harm justifying the entry of injunctive relief."); Appx1939-1940 ("[L]oss of market share …[is an] accepted form[] of irreparable harm.").

a. Price erosion

As Amgen does not dispute, its launch of Kanjinti will cause price erosion by forcing Genentech to decrease the amount it can charge for Herceptin. *E.g.*, *Celsis,* 664 F.3d at 930 (collecting cases wherein price erosion constitutes irreparable harm). Specifically, Amgen expects to set Kanjinti's net price at a [redacted] discount to Herceptin through 2028. Appx1741; Appx1396(¶58).

To maintain customers, Genentech will have to lower its effective net price for Herceptin, add rebates, and adjust contracts, resulting in a significant loss of revenue. Appx1477(¶¶48-52); Appx1396(¶58).

That price erosion will be irreversible because Genentech will not be able to recoup loss with future, higher prices or reduced discounts following a final judgment of Amgen's infringement. Appx1411-1413(¶¶99-101); *see Sanofi-Synthelabo*, 470 F.3d at 1382 (irreparable harm due to "irreversible price erosion"); *see also Polymer Techs.*, 103 F.3d at 976 ("Requiring purchasers to pay higher prices after years of paying lower prices to infringers is not a reliable business option."). The harms from Amgen's entry would continue even if Amgen were later removed from the market because Genentech would be unable to raise prices to pre-entry levels. Appx1411-1413(¶¶99-101).

As described above, the specific harm to Genentech as a result of price erosion is difficult to quantify, because Genentech will have numerous complex responses to Amgen's entry, and the specific effects of Amgen's activity will be difficult to unravel from other market conditions. Appx1399-1400(¶¶65-67); *see Sanofi-Synthelabo*, 470 F.3d at 1382; *Hoffmann-La Roche Inc. v. Cobalt Pharms., Inc.*, 2010 WL 4687839, at *12-13 (D.N.J. Nov. 10, 2010). This is so even after other trastuzumab biosimilars enter the market, as isolating the impact of Kanjinti

as opposed to other biosimilars on the price of Herceptin will be even more complex. Appx1401-1402(¶¶70-72); *AstraZeneca*, 633 F.3d at 1061-1062.

b. Lost market share

Amgen's launch of Kanjinti undisputedly will reduce Genentech's market share, another form of irreparable harm. *See, e.g.*, *Abbott Labs.*, 544 F.3d at 1361-1362; *Purdue Pharma L.P. v. Boehringer Ingelheim GmbH*, 237 F.3d 1359, 1368 (Fed. Cir. 2001). Kanjinti is a direct competitor of Herceptin, and Amgen itself forecasts that it will capture [redacted] of the trastuzumab market (all of which Genentech currently holds). Appx1741.

It will be difficult for Genentech to recapture market share from Amgen. As Amgen's own documents confirm, [redacted] Appx1751-1752. Even if Amgen were later removed from the market, Genentech is unlikely to recapture its pre-entry market share. Appx1411(¶98). Kanjinti's launch is expected to prime the marketplace for other biosimilar entrants as well as for any subsequent re-launch of Kanjinti. Appx1401(¶69). Once other trastuzumab biosimilars enter the market, Genentech will continue to be irreparably harmed because the percentage of the loss of market share attributable to Amgen will become even harder to quantify or to fully remedy. Appx1401-1402(¶¶70-72).

c. Effect on other products

The irreparable injuries that Genentech will suffer as a result of Amgen's launch of its infringing product are not limited only to Herceptin but extend to other Genentech products as well.

First, Amgen's biosimilar launch would likely have an incalculable but material negative effect on the market for Genentech's Perjeta and Kadcyla breast-cancer therapies. Perjeta is approved for use with Herceptin and they are thought to have synergistic effects. Appx1467(¶11); Appx1469-1470(¶23). Kadcyla is used for certain patients who have already been treated with Herceptin and chemotherapy and has been newly approved as an alternative to Herceptin for some patients. Appx1467(¶11); Appx1470(¶25). Should Amgen launch Kanjinti, the price differential between Kanjinti and Perjeta or Kadcyla will be far higher than the differential between Kanjinti and Herceptin, which may undermine payers' and providers' view of the incremental benefits of these treatments over Kanjinti. Furthermore, Amgen's marketing efforts to encourage providers to prescribe Kanjinti will detract from Genentech's efforts to focus providers on the benefits of Perjeta and Kadcyla, each of which is at an important inflection point in its lifecycle. Appx1406-1409(¶¶85-89, 93). Those negative impacts defy calculation.

Second, Amgen's launch would likely cause lost sales and price erosion for two other Genentech biologic drugs, Avastin and Rituxan, which are facing threats of biosimilar competition. For example, the availability of Kanjinti would increase pressure for Genentech to provide price concessions for Avastin and Rituxan. Appx1410-1411(¶96); Appx1476(¶47); Appx1478(¶¶57, 63). These harms are difficult to quantify and therefore irreparable.

Third, Genentech is a research-based company, and Amgen's launch will hinder Genentech's ability to fund research and development for new therapies. Appx1469(¶20); Appx1419-1420(¶¶117-119). The consequences of those lost research opportunities are impossible to know or quantify and are thus irreparable. *See Bio-Technology Gen. Corp. v. Genentech, Inc.*, 80 F.3d 1553, 1566 (Fed. Cir. 1996) (affirming irreparable harm based in part on reductions to research and development spending).

d. Reputational harm

Amgen's pretrial launch will irreparably injure Genentech even after Genentech earns a judgment at trial. Indeed, as Amgen itself has recognized, once a biosimilar competitor has launched, the patentee cannot enforce its patents by removing the biosimilar product from the market without suffering reputational harm for being "portrayed as taking a medicine off the market." Appx1934-1935; Appx1413-1414(¶¶102-104); *see also* Appx1923-1924. That reputational injury

further supports entry of a preliminary injunction to maintain the status quo pending an adjudication on the merits of Genentech's patent infringement claims. *See Douglas Dynamics, LLC v. Buyers Prods. Co.*, 717 F.3d 1336, 1344-1345 (Fed. Cir. 2013) (harm to "perception in the marketplace by customers … and distributors" is irreparable).

2. Genentech's irreparable harm is connected to Amgen's infringement

Amgen's own actions confirm the nexus between its infringing inducement of the three-weekly dosing regimen claims and the irreparable harm to Genentech. *Apple Inc. v. Samsung Elecs. Co.*, 809 F.3d 633, 640 (Fed. Cir. 2015) ("*Apple IV*") (explaining that there must be "'some connection' between the harm alleged and the infringing acts"). As described above, two of the dosing patents were upheld as valid in IPRs. After these decisions, Amgen attempted to [redacted]. Appx1694-1695(179:5-14, 179:24-180:4). This course of conduct, driven by Amgen's understanding of market demand, is overwhelming proof of nexus. *See Apple IV*, 809 F.3d at 643 (market demand for infringing features and infringer's belief that infringing features were driver of sales "establishes a causal nexus").

The patented dosing regimens account for most of the indications on the Herceptin label and are used in connection with a substantial majority of Herceptin prescriptions. Appx1496-1497(¶32); Appx2325; Appx2335. Amgen's own calculations confirm that this dosing regimen accounts for up to [redacted] of Herceptin prescriptions. Appx1827; Appx2499; Appx1696-1697(198:21-199:1); Appx1699-1702(205:16-208:24). Thus, there is unquestionably a nexus between Amgen's infringement and Genentech's irreparable harm.

II. THE REMAINING EQUITABLE FACTORS OVERWHELMINGLY FAVOR INJUNCTIVE RELIEF

The district court did not reach the remaining factors bearing on Genentech's preliminary injunction request, except a cursory discussion of the public interest in a footnote. The only reasonable conclusion is that all factors overwhelmingly support granting injunctive relief.

A. Genentech Is Likely To Succeed On The Merits

The only evidence presented to the district court shows that Genentech is likely to succeed on the merits of showing that Amgen infringes valid claims of the dosing patents.

Amgen did not dispute [redacted] in opposition to Genentech's motion and expert declaration of Dr. Susan Tannenbaum outlining on an element-by-element basis Amgen's infringement of the dosing patent claims. Appx3759 ("[redacted]

[redacted]

[redacted].”); *see also* Appx1500-1507(¶¶41-63).

As to validity, at the preliminary injunction phase, “the very existence of the patent satisfies [the patentee’s] burden on validity.” *Purdue Pharma*, 237 F.3d at 1365. Amgen was therefore required to show “evidence of invalidity [that] is sufficiently persuasive that it is likely to overcome the presumption of patent validity.” *PPG Indus., Inc. v. Guardian Indus. Corp.*, 75 F.3d 1558, 1566 (Fed. Cir. 1996). Amgen barely even tried to carry this burden; it presented no expert evidence and merely recycled art and arguments already rejected by the PTAB under a lower standard of proof.

After a full trial on the merits, a three-judge panel of the PTAB expressly rejected the primary invalidity arguments Amgen made in the district court.[6] For example: Amgen argued that “long established modeling tools were available to estimate Herceptin dosing at higher levels and longer intervals” (Appx3760, Appx3762), but the PTAB found that the relative novelty of using antibodies to treat cancer and the “inherent uncertainty associated with using mathematical models to predict the pharmacokinetic behavior of antibodies” refuted that

[6] With one exception, Amgen relied on the same prior art that was already considered and rejected by the PTAB. The one additional reference raised by Amgen, like the prior art considered in the IPRs, only discloses weekly dosing and is therefore, at best, cumulative. Appx3760-3761; Appx4510-4535.

argument. Appx3994, Appx4004. Amgen argued that a skilled artisan would have relied upon "trivial mathematics" to predict that a once-every-three-week dosing regimen would work (Appx3762), but the PTAB found that the prior art did not support the conclusion that a simple, linear model could be used. Appx3996. And Amgen argued that "there was no correlation between shed HER2 antigen levels and response to Herceptin treatment" (Appx3763), but the PTAB found that the prior art "highlight[ed] the uncertainty caused by the presence of shed antigens on the pharmacokinetics of trastuzumab." Appx3997. Amgen cannot demonstrate a likelihood of success when its arguments have been conclusively rejected by the PTAB in multiple IPR proceedings. *See Oxford Immunotec Ltd. v. Qiagen, Inc.*, 271 F. Supp. 3d 358, 366-367 (D. Mass. 2017).

Furthermore, Amgen had ample opportunity to develop and present expert testimony but simply chose not to. There are five expert declarations—from three oncologists and two pharmacokineticists—relied on by the PTAB that have been publicly available since 2017, and Amgen's invalidity expert reports were due less than two weeks after it opposed Genentech's preliminary injunction motion. In a case like this involving a complex technology, expert testimony is critical to proof of obviousness. *See, e.g., Allergan, Inc. v. Barr Labs., Inc.*, 501 F. App'x 965, 972 (Fed. Cir. 2013) (nonprecedential). Amgen instead chose to rely on conclusory assertions. As a result, the only expert testimony before the district court was that

of Genentech's expert, Dr. George Grass, who explained that the experience and data available to skilled artisans failed to establish a reasonable expectation of success regarding the patented dosing regimens. *See* Appx4759-4768(¶¶53-77). Amgen cannot raise a substantial question of invalidity based upon attorney argument alone.

In sum, where Amgen has not carried its burden on invalidity and [redacted], this factor weighs strongly in favor of Genentech.

B. The Balance Of Hardships Favors Genentech

The balance of hardships factor "assesses the relative effect of granting or denying an injunction on the parties." *Apple IV*, 809 F.3d at 645; *accord Kos Pharms., Inc. v. Andrx Corp.*, 369 F.3d 700, 727 (3d Cir. 2004). This factor likewise favors Genentech.

If Genentech's request for an injunction is denied, it will be "requir[ed] to compete against its own patented invention, with the resultant [irreparable] harms." *Robert Bosch LLC v. Pylon Mfg. Corp.*, 659 F.3d 1142, 1156 (Fed. Cir. 2011). If Amgen is allowed to continue its launch, Genentech will suffer price erosion and loss of market share, with no possibility that it can be made whole after trial.

By comparison, Amgen itself has acknowledged that it will not suffer harm by delaying a launch. Amgen's own projections state it [redacted]

(Appx1977), and no other trastuzumab biosimilar will launch before [redacted] (Appx1476(¶45)).

To the extent Amgen asserts that having to stop its launch will harm its reputation, "an alleged infringer's loss of market share and customer relationships, without more, does not rise to the level necessary to overcome the loss of exclusivity experienced by a patent owner due to infringing conduct." *Pfizer*, 429 F.3d at 1382. Genentech informed Amgen that it intended to continue to seek preliminary injunctive relief—including an injunction pending resolution of this appeal—immediately after the district court denied Genentech's motions for a temporary restraining order and preliminary injunction. Appx4945(32:5-10); Appx4950(37:6-15). Amgen nevertheless issued a press release several hours later announcing that Kanjinti is now available in the United States. ECF No. 9, Ex. 1. Any harm attributable to Amgen's decision to proceed with launch in the face of Genentech's injunction request is self-inflicted and does not favor Amgen under the balance of hardships. *Celsis*, 664 F.3d at 931 (district court did not err in finding balance of harms in patent holder's favor where "the preliminary record suggests that [infringer's] losses were the result of its own calculated risk in selling a product with knowledge of Celsis' patent"); *Sanofi-Synthelabo*, 470 F.3d at 1383 (balance of hardships favored the patent holder where the harms asserted by the infringing party "were the result of its own calculated risk to launch its product

pre-judgment"). Indeed, Amgen itself has argued that where, as here, the infringer acts at risk, any hardship attributable to the infringer of ceasing its conduct cannot support the infringer under the balance of hardships. Appx4875-4878 (16-19 & n.10).

C. The Public Interest Is Served By Enforcing Genentech's Patent Rights To Encourage Innovation

The last factor for the Court to consider is the public interest. In the preliminary-injunction context, this factor focuses "on whether a critical public interest would be injured by the grant of injunctive relief." *Metalcraft of Mayville, Inc. v. Toro Co.*, 848 F.3d 1358, 1369 (Fed. Cir. 2017). Here, the public interest is best served by "the enforcement of [Genentech's] patent rights." *Celsis*, 664 F.3d at 931-932; Appx1416-1421(¶¶111-124). As this Court has explained, "investment in drug research and development must be encouraged and protected by the exclusionary rights conveyed in valid patents." *Celsis*, 664 F.3d at 931; *see also Sanofi-Synthelabo*, 470 F.3d at 1383-1384.

Amgen did not dispute that the public interest favors the enforcement of patent rights to encourage innovation—a position that, once again, Amgen itself has taken in other cases. *See, e.g.*, Appx1924-1925 (("The Public Has a Strong Interest in a Robust Patent System that Maintains the Incentives for Pharmaceutical Innovation"); Appx1935 (("There is a strong public interest in encouraging investment in the research and development to create novel biological therapeutics

that treat human disease. The fact that a copyist may sell at a lower price does not override this important public interest."). Nor does Amgen contend that an injunction will adversely affect patient care. Rather, Amgen argued, and the district court agreed in a footnote, that an injunction would deprive the public of access to non-infringing uses of Kanjinti. Appx3765; Appx9-10 n.7 (quoting *Apple III*, 735 F.3d at 1372-1373). But by Amgen's own estimation, [redacted] of patient use is covered by Genentech's patents. Appx1827. And if Amgen were truly concerned with public access to ***non-infringing*** uses of Kanjinti, it could have tried to eliminate the infringing indications from its label—[redacted] *See supra* pp. 10-11. Moreover, neither Amgen nor the district court has pointed to any patient need for the non-infringing uses that cannot already be supplied by Herceptin, which Genentech is fully able to supply.

CONCLUSION

Genentech respectfully requests that the Court reverse the district court's denial of a preliminary injunction, or alternatively vacate and remand for further consideration under the correct legal standards. Should the Court grant Genentech's pending motion under Fed. R. App. P. 8 and subsequently remand for the district court for further consideration, Genentech respectfully requests that this

Court order that the injunction pending appeal continue through the district court's determination on remand.

Respectfully submitted,

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July 26, 2019

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IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF DELAWARE

GENENTECH, INC. and CITY OF HOPE, *Plaintiffs*, v. AMGEN INC., *Defendant.*	Civ. No. 18-924-CFC

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MEMORANDUM OPINION

July 18, 2019

CONNOLLY, UNITED STATES DISTRICT JUDGE

This action arises under the Biologics Price Competition and Innovation Act of 2009 (BPCIA), Pub. L. No. 111–148, §§ 7001–7003, 124 Stat. 119, 804–21 (2010) (codified as amended at 42 U.S.C. § 262, 35 U.S.C. § 271(e), 28 U.S.C. § 2201(b), 21 U.S.C. § 355 et seq.). Plaintiffs Genentech, Inc. and City of Hope have sued Defendant Amgen Inc. based on Amgen's submission of a Biologics License Application (BLA) for approval to market Kanjinti, a biosimilar of Genentech's drug product Herceptin.

On May 15, 2018, Amgen served Genentech a Notice of Commercial Marketing pursuant to § 262(*l*)(8)(A) of the BPCIA. Kanjinti was approved by the FDA on June 13, 2019. Four weeks later, on July 10, 2019, Genentech moved for a temporary restraining order and preliminary injunction to prevent Amgen from commercially launching, marketing, or selling Kanjinti until the Court renders a decision on the merits of Genentech's patent infringement claims following trial, and until the Court of Appeals for the Federal Circuit has adjudicated any appeal of that decision. D.I. 273; D.I. 274. That same day, I arranged an emergency teleconference with the parties and orally ordered a standstill until I received Amgen's response to Genentech's motions and had an opportunity to consider

fully the issues and rule on the merits. For the foregoing reasons, I will deny Genentech's motions for a temporary restraining order and preliminary injunction.

I. BACKGROUND

The non-proprietary names for Herceptin and Kanjinti are respectively trastuzumab and trastuzumab-anns.[1] For purposes of a trial scheduled for December 2019, the parties are litigating ten patents which cover: (i) the trastuzumab antibody itself (the Composition Patent)[2]; (ii) techniques for identifying patients who might benefit from trastuzumab therapy (the HER2 Diagnostic Patents)[3]; (2) various aspects of cell culture, purification, and antibody manufacturing purification (the Manufacturing Patents)[4]; and (3) methods of administration (the Dosing Patents). D.I. 44; D.I. 60 at 2-3; D.I. 75. Genentech's motions seek relief based on claims in the three Dosing Patents: U.S. Patent Nos. 6,627,196 (the "#196 patent"), 7,371,379 (the "#379 patent") and 10,160,811 (the "#811 patent"). All three patents relate to methods of treating cancer with a

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[1] The FDA employs a "naming convention" pursuant to which it gives a "core name" to the reference product (in this case, trastuzumab) and adds for each biosimilar a "distinguishing suffix that is devoid of meaning and composed of four lowercase letters ... attached with a hyphen to the core name" (in this case, "-anns").

[2] U.S. Patent No. 6,407,213 claims the trastuzumab antibody.

[3] The HER2 Diagnostic Patents at issue are U.S. Patent Nos. 7,993,834 and 8,076,066.

[4] The Manufacturing Patents at issue are U.S. Patent Nos. 6,620,918; 8,512,983; 8,574,869; and 9,714,293.

specific dosage regimen: intravenous ("IV") administration of an initial 8 mg/kg dose followed by one or more 6 mg/kg doses separated by three weeks. D.I. 279-1, Ex. 1, Cl. 11; Ex. 2, Cl. 11; Ex. 3, Cl. 6. The #379 patent further recites coadministration with a chemotherapy agent. *Id.*, Ex. 2, Cl. 6. The #811 patent further recites treatment of breast cancer. *Id.*, Ex. 3, Cl. 11.

II. LEGAL STANDARDS

A preliminary injunction is "a drastic and extraordinary remedy that is not to be routinely granted." *Intel Corp. v. ULSI Sys. Tech., Inc.*, 995 F.2d 1566, 1568 (Fed. Cir. 1993). To obtain such extraordinary relief, the moving party must prove that: (1) it has a reasonable likelihood of success on the merits; (2) it would suffer irreparable harm in the absence of an injunction; (3) the balance of hardships tips in its favor; and (4) an injunction would have a favorable impact on the public interest. *Amazon.com, Inc. v. Barnesandnoble.com, Inc.*, 239 F.3d 1343, 1350 (Fed. Cir. 2001). "These factors, taken individually, are not dispositive; rather, the district court must weigh and measure each factor against the other factors and against the form and magnitude of the relief requested." *Hybritech Inc. v. Abbott Lab.*, 849 F.2d 1446, 1451 (Fed. Cir. 1988). The grant or denial of a preliminary injunction is within the sound discretion of the district court. *Polymer Tech., Inc. v. Bridwell*, 103 F.3d 970, 973 (Fed. Cir. 1996).

The standards for a preliminary injunction also apply to a motion for a temporary restraining order when, as here, the opposing party has notice of the motion. *See Takeda Pharm. USA, Inc. v. W.-Ward Pharm. Corp.*, 2014 WL 5088690, at *1 (D. Del. Oct. 9, 2014). Accordingly, Genentech's motion for a temporary restraining order rises and falls with its motion for a preliminary injunction.

III. DISCUSSION

"Central to the movant's burden are the likelihood of success and irreparable harm factors." *Sofamor Danek Grp., Inc. v. DePuy-Motech, Inc.*, 74 F.3d 1216, 1219 (Fed. Cir. 1996). "A court may decline to issue a preliminary injunction if the movant does not prove either of these factors." *Jeneric/Pentron, Inc. v. Dillon Co.*, 205 F.3d 1377, 1380 (Fed. Cir. 2000). Here, I am denying the motion for preliminary injunction, because Genentech has failed to establish irreparable harm.

A patentee's undue delay in seeking a preliminary injunction "negates the idea of irreparability." *Pfizer, Inc. v. Teva Pharm., USA, Inc.*, 429 F.3d 1364, 1382 (Fed. Cir. 2005); *Polymer Tech.*, 103 F.3d at 974 (same). Genentech has known of Amgen's intent to market Kanjinti since Amgen served its 180-day Notice of Commercial Marketing on May 15, 2018. In addition, Genentech received information through discovery that made clear Amgen's plan to launch its

marketing of Kanjinti in July 2019. Specifically, in February 2019, Amgen produced to Genentech documents showing that it filed a "resubmission" to the FDA in December 2018.[5] Given the known six-month regulatory timeline for the FDA to consider the resubmission (*see* D.I. 289-1, Ex. 11 at 4), Genentech would have understood at the time that the FDA would act on the resubmission by the end of June 2019. In April 2019, Amgen produced documents with its launch plan redactions removed, thus enabling Genentech to see that Amgen planned to launch in July 2019. *Id.* at Ex. 12. From late April through mid-June, five Amgen witnesses testified during depositions that Amgen was preparing to be ready to launch Kanjinti in July 2019. D.I. 289-1, Ex. 14 at 66:12-67:3, 83:9-12; Ex. 15 at 40:20-23; Ex. 16 at 79:6-10, 81:3-6; Ex. 17 at 18:5-10; and Ex. 18 at 32:11-33:18.

The FDA approved Kanjinti on June 13, 2019. But Genentech did not file its motion for a preliminary injunction until July 10, 2019—fourteen months after receiving the Notice of Commercial Marketing, three months after receiving a fairly specific launch date, and almost one month after Amgen had FDA approval to launch Kanjinti.

[5] A resubmission is "a submission by the biologics license applicant or supplement applicant of all materials needed to fully address all deficiencies identified in the complete response letter." 21 C.F.R. § 600.3.

Genentech's actions are also contrary to the spirit and purpose of the BPCIA. As the Federal Circuit explained, the 180-day period triggered by the notice of commercial marketing "gives the parties and the district court the time for adjudicating such matters without the reliability-reducing rush that would attend requests for relief against immediate market entry that could cause irreparable injury." *Amgen Inc. v. Apotex Inc.*, 827 F.3d 1052, 1063 (Fed. Cir. 2016), *cert. denied*, 137 S. Ct. 591 (2016). Thus, the 180-day period is designed to prevent exactly the circumstances that Genentech has engineered in this case—a "race to court for immediate relief to avoid irreparable harm from market entry, and ... the hurried motion practice that (8)(A) is designed to replace."[6] *Id.* at 1065.

Genentech's undue delay in requesting a preliminary injunction, particularly in light of relevant provisions under the BPCIA, should be sufficient by itself to deny the motion. Nevertheless, a finding of no irreparable harm is also supported by the fact that Genentech has engaged in a pattern and practice of licensing the Dosage Patents.

[6] Notably, Genentech demonstrated its ability to avoid hurried motion practice in a related case involving the same patents and the same reference product but a different defendant. With Amgen, however, Genentech represented to the Court as recently as May 16, 2019 that it was not seeking a preliminary injunction. *See* D.I. 289-1, Ex. 19 at 26:1-4.

An injunction is a form of equitable relief and, therefore, available only when there is no adequate remedy at law. *See N. Cal. Power Agency v. Grace Geothermal Corp.*, 105 S.Ct. 459, 459 (1984) ("A party seeking an injunction from a federal court must invariably show that it does not have an adequate remedy at law."); *Coca-Cola Bottling Co. of Shreveport, Inc. v. Coca-Cola Co.*, 769 F. Supp. 671, 713 (D. Del. 1991) ("The Court may only invoke its equity powers when there is no adequate remedy at law."). Thus, to establish irreparable harm, the movant must "clearly establish[] that monetary damages could not suffice." *Abbott Labs. v. Andrx Pharm., Inc.*, 452 F.3d 1331, 1348 (Fed. Cir. 2006). The fact that "movants have engaged in a pattern of granting licenses under the patent" makes it "reasonable to expect that invasion of the patent right can be recompensed with a royalty rather than with an injunction." *Polymer Tech.*, 103 F.3d at 974; *see also High Tech Med. Instrumentation, Inc. v. New Image Indus., Inc.*, 49 F.3d 1551, 1557 (Fed. Cir. 1995) (offering to license the patent "suggests that any injury suffered by [the patentee] would be compensable in damages"). Here, Genentech granted [redacted] for the Dosing Patents to Mylan, Celltrion, and Pfizer that allow a biosimilar to enter the market in [redacted]. D.I. 291-1, Exs. 37-39. In other words, Genentech has been able to place a value on the patents and has approved competitors entering the

market [redacted]. Under these facts, any potential damages for sales in the next four months should be quantifiable. *See King Pharm., Inc. v. Sandoz, Inc.*, 2010 WL 1957640, at *6 (D.N.J. May 17, 2010) (denying a preliminary injunction where any changes to the market from the non-movant's entry should be easy to calculate given the short period time).

The "absence of irreparable harm ... ma[kes] unnecessary a consideration of ... [the] likelihood of success in proving infringement." *Ill. Tool Works, Inc. v. Grip-Pak, Inc.*, 906 F.2d 679, 682 n.3 (Fed. Cir. 1990); *Polymer Tech.*, 103 F.3d at 974 ("[T]he district court did not err by focusing its analysis solely on irreparable harm in denying [the movant's] motion."). Due to the hurried nature of this particular motion practice, I will not take additional time to set forth my analysis with respect to other preliminary injunction factors.[7] Genentech has failed to

[7] I will briefly make note of considerations under the fourth factor that also weigh in favor of denying the motion for a preliminary injunction. "[A]lthough there exists a public interest in protecting rights secured by valid patents, the focus of the district court's public interest analysis should be whether there exists some critical public interest that would be injured by the grant of preliminary relief." *Hybritech Inc.*, 849 F.2d at 1458. For pharmaceutical drugs that prolong and save lives, there is a critical public interest in affordable access to those drugs. Genentech itself acknowledges this public interest by stating that it is "committed to ensuring patient access by providing Herceptin free of charge to patients who are uninsured or cannot afford treatment." D.I. 275 at 18. In that context, I note that Genentech's exclusivity based on the Composition Patent expired on June 18, 2019, and only two of the four indications on the Kanjinti label allegedly infringe the Dosing Patents, meaning there are two recited methods of using Kanjinti that

establish irreparable harm and therefore its motions for preliminary and temporary injunctive relief must be denied.

IV. CONCLUSION

For the foregoing reasons, I will deny Genentech's motions for a temporary restraining order and preliminary injunction (D.I. 273; D.I. 274). The standstill ordered on July 10, 2019 is lifted.

The Court will issue an Order consistent with this Memorandum Opinion.

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are free of any allegations of infringement. "[T]he prospect that an injunction would have the effect of depriving the public of access to a large number of non-infringing features," weighs against granting an injunction. *Apple Inc. v. Samsung Elecs. Co. Ltd.*, 735 F.3d 1352, 1372–73 (Fed. Cir. 2013).

IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF DELAWARE

GENENTECH, INC. and CITY OF HOPE, *Plaintiffs*, v. AMGEN INC., *Defendant*.	Civ. No. 18-924-CFC

ORDER

IT IS HEREBY ORDERED, for the reasons stated in the accompanying Memorandum, that:

1. Genentech's motion for a temporary restraining order (D.I. 273) is DENIED;

2. Genentech's motion for a preliminary injunction (D.I. 274) is DENIED; and

3. The standstill order given during the July 10, 2019 teleconference is lifted.

Dated: July 18, 2019

UNITED STATES DISTRICT JUDGE

US006627196B1

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

(10) **Patent No.: US 6,627,196 B1**
(45) **Date of Patent: Sep. 30, 2003**

(54) **DOSAGES FOR TREATMENT WITH ANTI-ERBB2 ANTIBODIES**

(75) Inventors: **Sharon A. Baughman**, Ventura, CA (US); **Steven Shak**, Burlingame, CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/648,067**

(22) Filed: **Aug. 25, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/213,822, filed on Jun. 23, 2000, and provisional application No. 60/151,018, filed on Aug. 27, 1999.

(51) **Int. Cl.**[7] **A61K 39/395**

(52) **U.S. Cl.** **424/138.1**; 424/131.1; 424/133.1; 424/134.1; 424/135.1; 424/136.1; 424/137.1; 424/139.1; 424/141.1; 424/142.1; 424/143.1; 424/144.1; 424/145.1; 424/146.1; 424/147.1; 424/150.1; 424/151.1; 424/152.1; 424/153.1; 424/154.1; 424/155.1; 424/156.1; 424/158.1; 424/172.1; 424/174.1

(58) **Field of Search** 424/130.1, 138.1, 424/141.1, 142.1, 152.1, 155.1, 131.1, 133.1, 134.1, 135.1, 136.1, 137.1, 139.1, 143.1, 144.1, 145.1, 146.1, 147.1, 150.1, 151.1, 153.1, 154.1, 156.1, 158.1, 172.1, 174.1

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(57) **ABSTRACT**

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.

33 Claims, 5 Drawing Sheets

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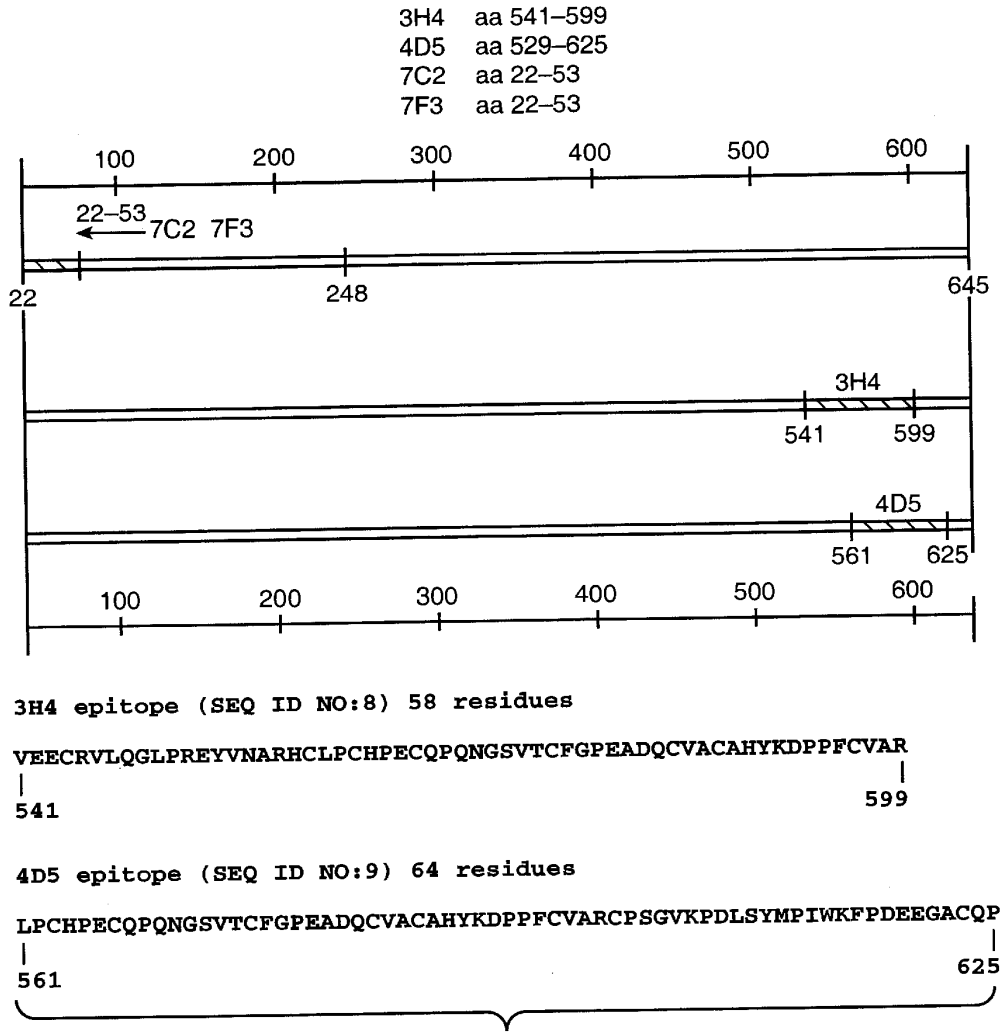

3H4 epitope (SEQ ID NO:8) 58 residues

VEECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVAR

541 **599**

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

LPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQP

561 **625**

FIG._1

1 MELAALCRWGLLLALLPPGAA**STQVCTGTDMKLRLPA**

38 **SPETHLDMLRHLYQGC**QVVQGNLELTYLPTNASLSFL

75 QDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDN

112 YALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEI

149 LKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLID

186 TNRSRA

FIG._2

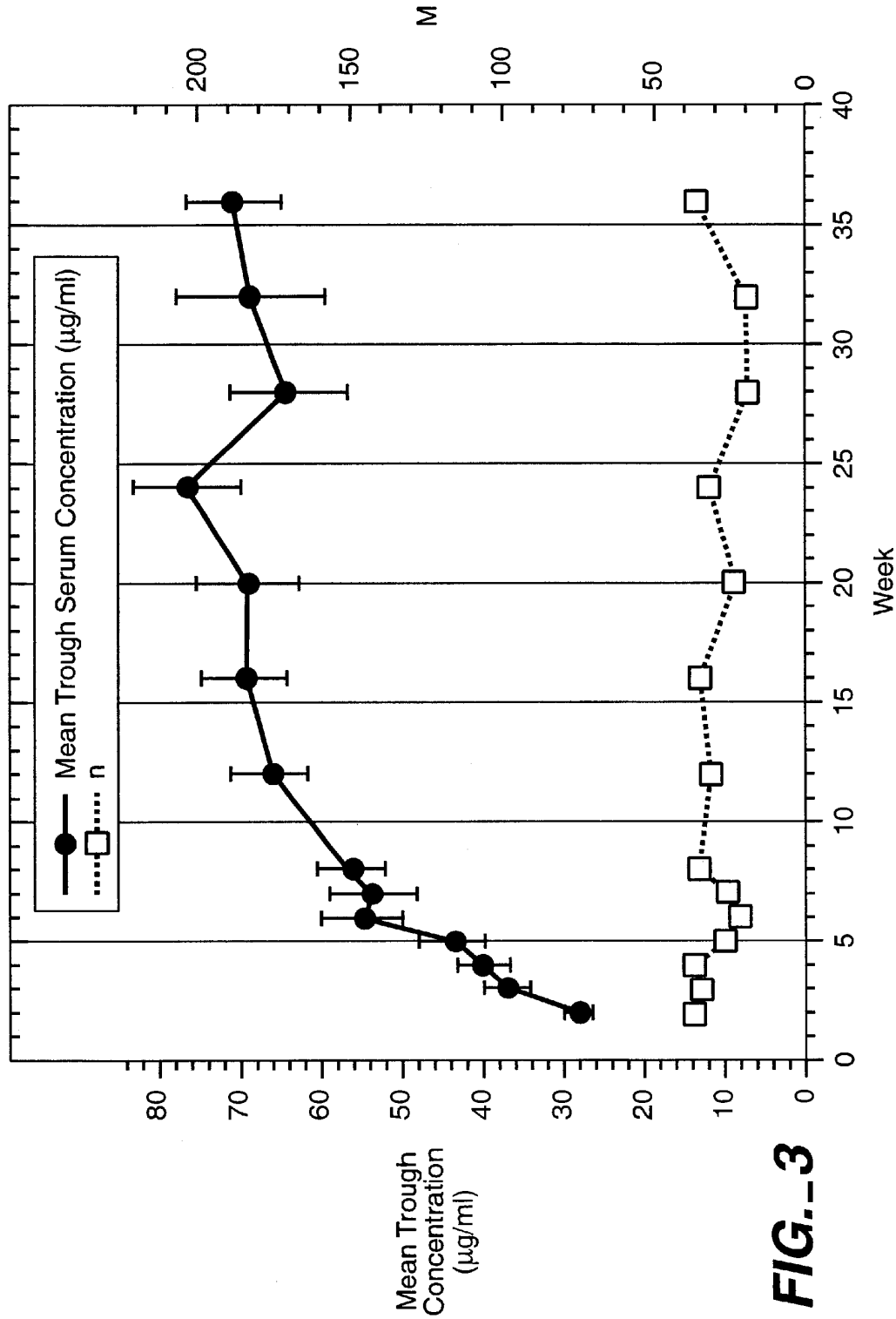
Mean Trough Serum Concentration (µg/ml)
n
Mean Trough Concentration (µg/ml)
Week
M
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25
30
35
40
0
50
100
150
200

FIG._3

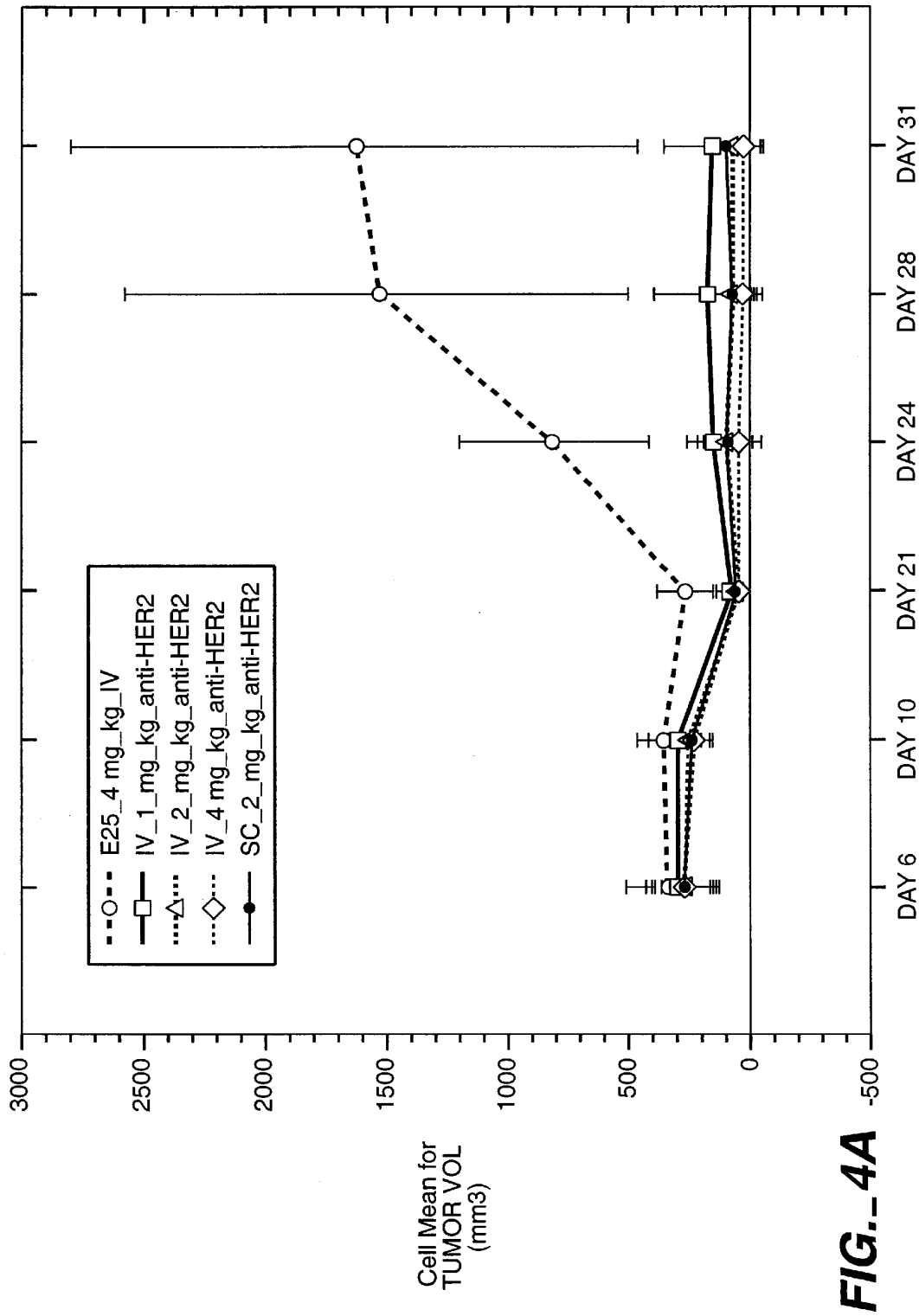
E25_4 mg_kg_IV
IV_1_mg_kg_anti-HER2
IV_2_mg_kg_anti-HER2
IV_4 mg_kg_anti-HER2
SC_2_mg_kg_anti-HER2
Cell Mean for TUMOR VOL (mm3)
3000
2500
2000
1500
1000
500
0
-500
DAY 6
DAY 10
DAY 21
DAY 24
DAY 28
DAY 31

FIG._4A

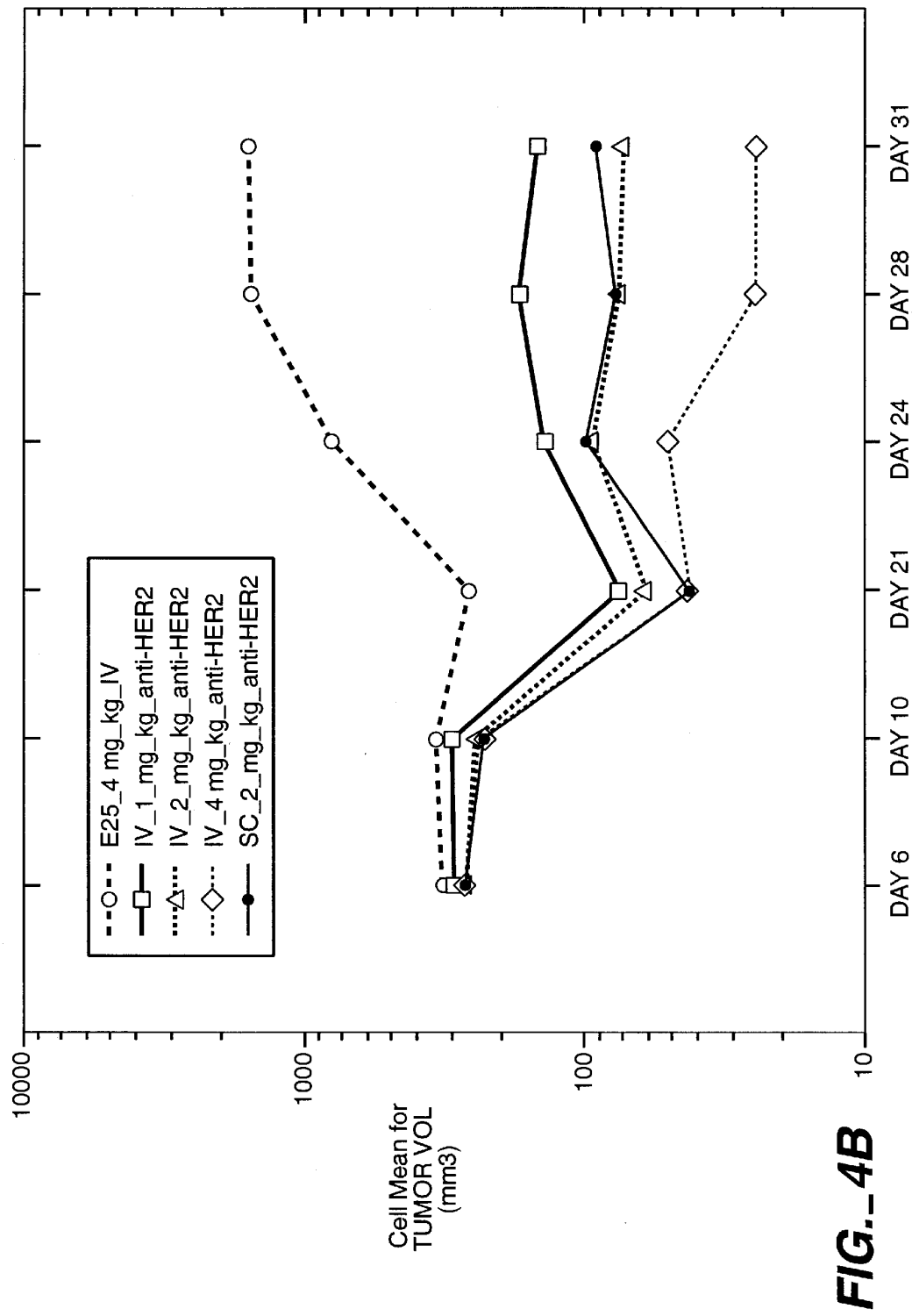
E25_4 mg_kg_IV
IV_1_mg_kg_anti-HER2
IV_2_mg_kg_anti-HER2
IV_4 mg_kg_anti-HER2
SC_2_mg_kg_anti-HER2
Cell Mean for TUMOR VOL (mm3)
10000
1000
100
10
DAY 6
DAY 10
DAY 21
DAY 24
DAY 28
DAY 31

FIG._4B

VARIABLE LIGHT

```
          1       10        20          30              40
2C4       DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIGVA----] WYQQRP
           **    **** *      *                        *
574       DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA----] WYQQKP
                                   *   *  ****
hum kI    DIQMTQSPSSLSASVGDRVTITC [RASQSVSTSSYSYMH] WYQQKP

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

                   90          100
2C4       EDLAVYYC [QQYYIYPYT] FGGGTKLEIK (SEQ ID NO:10)
            * *                  *   *
574       EDFATYYC [QQYYIYPYT] FGQGTKVEIK (SEQ ID NO:12)
                       ***
hum kI    EDFATYYC [QQYNSLPYT] FGQGTKVEIK (SEQ ID NO:14)
```

FIG._5A

VARIABLE HEAVY

```
          1       10        20          30          40
2C4       EVQLQQSGPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS
              **  **  *  * ***  **                   *
574       EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA
                                       ** * *
humIII    EVQLVESGGGSVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                    50        60          70        80
2C4       HGKSLEWIG [DVNPNSGGSIYNQRFKG] KASLTVDRSSRIVYM
          *  *   **                     *** *    **** *
574       PGKGLEWVA [DVNPNSGGSIYNQRFKG] RFTLSVDRSKNTLYL
                  *  ******  ** ****       * * *
humIII    PGKGLEWVS [VISGDGGSTYYADSVKG] RFTISRDDSKNTLYL

                     90         100          110
2C4       ELRSLTFEDTAVYYCAR [NLGPSFYFDY] WGQGTTLVTSS (SEQ ID NO:11)
          ***  **                                *
574       QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS (SEQ ID NO:13)
                            ** ***
humIII    QMNSLRAEDTAVYYCAR [GRGGGS--DY] WGQGTLVTVSS (SEQ ID NO:15)
```

FIG._5B

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 ($p185^{HER2}$) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer (Slamon et al., *Science* 235:177–182 [1987]; Slamon et al., *Science* 244:707–712 [1989]).

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

Antibodies directed against human erbB2 protein products and proteins encoded by the rat equivalent of the erbB2 gene (neu) have been described. Drebin et al., *Cell* 41:695–706 (1985) refer to an IgG2a monoclonal antibody which is directed against the rat neu gene product. This antibody called 7.16.4 causes down-modulation of cell surface p185 expression on B104-1-1 cells (NIH-3T3 cells transfected with the neu proto-oncogene) a inhibits colony formation of these cells. In Drebin et al. *PNAS* (*USA*) 83:9129–9133 (1986), the 7.16.4 antibody was shown to

inhibit the tumorigenic growth of neu-transformed NIH-3T3 cells as well as rat neuroblastoma cells (from which the neu oncogene was initially isolated) implanted into nude mice. Drebin et al. in *Oncogene* 2:387–394 (1988) discuss the production of a panel of antibodies against the rat neu gene product. All of the antibodies were found to exert a cytostatic effect on the growth of neu-transformed cells suspended in soft agar. Antibodies of the IgM, IgG2a and IgG2b isotypes were able to mediate significant in vitro lysis of neu-transformed cells in the presence of complement, whereas none of the antibodies were able to mediate high levels of antibody-dependent cellular cytotoxicity (ADCC) of the neu-transformed cells. Drebin et al. *Oncogene* 2:273–277 (1988) report that mixtures of antibodies reactive with two distinct regions on the p185 molecule result in synergistic anti-tumor effects on neu-transformed NIH-3T3 cells implanted into nude mice. Biological effects of anti-neu antibodies are reviewed in Myers et al., *Meth. Enzym.* 198:277–290 (1991). See also WO94/22478 published Oct. 13, 1994.

Hudziak et al., *Mol. Cell. Biol.* 9(3):1165–1172 (1989) describe the generation of a panel of anti-ErbB2 antibodies which were characterized using the human breast tumor cell line SKBR3. Relative cell proliferation of the SKBR3 cells following exposure to the antibodies was determined by crystal violet staining of the monolayers after 72 hours. Using this assay, maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel,including 7C2 and 7F3, reduced cellular proliferation to a lesser extent in this assay. Hudziak et al. conclude that the effect of the 4D5 antibody on SKBR3 cells was cytostatic rather than cytotoxic, since SKBR3 cells resumed growth at a nearly normal rate following removal of the antibody from the medium. The antibody 4D5 was further found to sensitize p 185 -overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also WO89/06692 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); Vitetta et al. *Cancer Research* 54:5301–5309 (1994); Sliwkowski et al. *J. Biol. Chem.* 269(20): 14661–14665 (1994); Scott et al. *J. Biol. Chem.* 266:14300–5 (1991); and D'souza et al. *Proc. Natl. Acad. Sci.*91:7202–7206 (1994).

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

McKenzie et al. *Oncogene* 4:543–548 (1989) generated a panel of anti-ErbB2 antibodies with varying epitope specificities, including the antibody designated TA1. This TA1 antibody was found to induce accelerated endocytosis of ErbB2 (see Maier et al. *Cancer Res.* 51:5361–5369 [1991]). Bacus et al. *Molecular Carcinogenesis* 3:350–362 (1990) reported that the TA1 antibody induced maturation of the breast cancer cell lines AU-565 (which overexpresses the 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 fibroblasts 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 fibroblasts overexpressing human ErbB2 was assessed. As observed in their earlier work, N28 accelerated tumor growth, whereas N12 and N29 significantly inhibited growth of the ErbB2-expressing cells. Partial tumor inhibition was also observed with the N24 antibody. Bacus et al. also tested the ability of the antibodies to promote a mature phenotype in the human breast cancer cell lines AU-565 and MDA-MB453 (which overexpress-ErbB2) as well as MCF-7 (containing low levels of the receptor). Bacus et al. saw a correlation between tumor inhibition in vivo and cellular differentiation; the tumor-stimulatory antibody N28 had no effect on differentiation, and the tumor inhibitory action of the N12, N29 and N24 antibodies correlated with the extent of differentiation they induced.

Xu et a. *Int. J. Cancer* 53:401–408 (1993) evaluated a panel of anti-ErbB2 antibodies for their epitope binding specificities, as well as their ability to inhibit anchorage-independent and anchorage-dependent growth of SKBR3 cells (by individual antibodies and in combinations), modulate cell-surface ErbB2, and inhibit ligand stimulated anchorage-independent growth. See also WO94/00136 published Jan 6, 1994 and Kasprzyk et al. *Cancer Research* 52:2771–2776 (1992) concerning anti-ErbB2 antibody combinations. Other anti-ErbB2 antibodies are discussed in Hancock et al. *Cancer Res.* 51:4575–4580 (1991); Shawver et al. *Cancer Res.* 54:1367–1373 (1994); Arteaga et al. *Cancer Res.* 54:3758–3765 (1994); and Harwerth et al. *J. Biol. Chem.* 267:15160–15167 (1992).

A recombinant humanized anti-ErbB2 monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2, 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 (Baselga et al., *J. Clin Oncol.* 14:737–744 [1996]). The recommended initial loading dose for HERCEPTIN®O 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 Chamness, *Gene* 159:19–27 [1995]; and Hynes and Stern, *Biochim Biophys Acta* 1198:165–184 [1994]), and has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluoruracil) and anthracyclines (Baselga et al., *Oncology* 11 (3 Suppl 1):43–48 [1997]). However, despite the association of ErbB2 overexpression with poor prognosis, the odds of HER2-positive patients responding clinically to treatment with taxanes were greater than three times those of HER2-negative patients (Ibid). rhuMab HER2 was shown to enhance the activity of paclitaxel (TAXOL®) and doxorubicin against breast cancer xenografts in nude mice injected with BT-474 human breast adenocarcinoma cells, which express high levels of HER2 (Baselga et al., *Breast Cancer, Proceedings of ASCO*, Vol. 13, Abstract 53 [1994]).

SUMMARY OF THE INVENTION

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 μg/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 of8 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 o 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 about 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. Generally, two or more subsequent doses (e.g. from about two to about ten subsequent doses) of the antibody are administered to the patient, and those subsequent doses are preferably 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). The two or more subsequent doses may each be from about 2 mg/kg to about 16 mg/kg; or from about 4 mg/kg to about 12 mg/kg; or from about 6 mg/kg to about 12 mg/kg. 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.

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. The invention additionally provides an article of manufacture, comprising a container, a composition within the container comprising an anti-ErbB 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 label on or associated with the container that indicates that the composition can be used for treating a condition characterized by overexpression of ErbB2 receptor, and a package insert containing instructions to avoid the use of anthracycline-type chemotherapeutics in combination with the composition. According to the invention, the 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 including, but not limited to, paclitaxel and docetaxel. In another embodiment, the chemotherapeutic agent is an anthracyline derivative including, but not limited to, doxorubicin or epirubicin. In still another embodiment of the invention, treatment with an anti-ErbB2 antibody and an anthracycline derivative further includes administration of 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 composition and a chemotherapeutic agent to a patient. In another embodiment, the chemotherapeutic agent is other than an anthracycline, and is preferably a taxoid, such as TAXOL®. In still another embodiment, the chemotherapeutic agent is an anthracycline, including but not limited to, doxorubicin or epirubicin. In yet another embodiment, the chemotherapeutic agent is an anthracycline and the package insert further instructs the user to administer a cardioprotectant.

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 huMAb4D5–8 (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, $F(ab')_2$, diabody, and the like). The variable light chain and variable heavy chain regions of humanized anti-ErbB2 antibody 2C4 are shown in FIGS. **5**A and **5**B.

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 *J. Cell Biol.* 125(6):1395–1406 [June 1994]). The anti-proliferative MAbs 4D5 and 3H4 b bind adjacent to the transmembrane domain. The various ErbB2-ECD truncations or point mutations were prepared from cDNA using polymerase chain reaction technology. The ErbB2 mutants were expressed as gD fusion proteins in a mammalian expression plasmid. This expression plasmid uses the cytomegalovirus promoter/enhancer with SV40 termination and polyadenylation signals located downstream of the inserted cDNA. Plasmid DNA was transfected into 293S cells. One day following transfection, the cells were metabolically labeled overnight in methionine and cysteine-free, low glucose DMEM containing 1% dialyzed fetal bovine serum and 25 μCi each of ^{35}S methionine and ^{35}S cysteine. Supernatants were harvested either the ErbB2 MAbs or control antibodies were added to the supernatant and incubated 2–4 hours at 4° C. The complexes were precipitated, applied to a 10–20% Tricine SDS gradient gel and electrophoresed at 100 V. The gel was electroblotted onto a membrane and analyzed by autoradiography. SEQ ID NOs:8 and 9 depict the 3H4 and 4D5 epitopes, respectively.

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

FIG. **3** is a graph of anti-ErbB2 antibody (HERCEPTIN®) trough serum concentration (μg/ml, mean ±SE, dark circles) by week from week 2 through week 36 for ErbB2 overexpressing patients treated with HERCEPTIN® anti-ErbB2 antibody at 4 mg/kg initial dose, followed by 2 mg/kg weekly. The number of patients at each time point is represented by "n" (white squares).

FIG. **4**A is a linear plot of tumor volume changes over time in mice treated with HERCEPTIN® anti-ErbB2 antibody. FIG. **4**B is a semi-logarithmic plot of the same data as in FIG. **4**A such that the variation in tumor volume for the treated animals is observed more readily.

FIGS. **5**A and **5**B depict alignments of the amino acid sequences of the variable light (V_L)(FIG. **5**A) and variable heavy (V_H) (FIG. **5**B) domains of murine monoclonal antibody 2C4 (SEQ ID Nos. 10 and 11, respectively); V_L and V_H domains of humanized Fab version 574 (SEQ ID Nos. 12 and 13, respectively), and human V_L and V_H consensus frameworks (hum κl, light kappa subgroup I; humIII, heavy subgroup III) (SEQ ID Nos. 14 and 15, respectively). Asterisks identify differences between humanized Fab version 574 and murine monoclonal antibody 2C4 or between humanized Fab version 574 and the human framework. Complementarity Determining Regions (CDRs) are in brackets. Humanized Fab version 574, with the changes ArgH71Val, AspH73Arg and IleH69Leu, appears to have binding restored to that of the original chimeric 2C4 Fab fragment. Additional FR and/or CDR residues, such as L2, L54, L55, L56, H35 and/or H48, may be modified (e.g. substituted as follows-IleL2Thr; ArgL54Leu; TyrL55Glu; ThrL56Ser; AspH35Ser; and ValH48Ile) in order to further refine or enhance binding of the humanized antibody. Alternatively, or additionally, the humanized antibody may be affinity matured in order to further improve or refine its affinity and/or other biological activities.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

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. *Ann. Rev. Biochem.* 56:881–914 (1987), including variants thereof (e.g. a deletion mutant EGFR as in Humphrey et al. *PNAS (USA)* 87:4207–4211 (1990)). erbB1 refers to the gene encoding the EGFR protein product. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL RB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, U.S. Pat. No. 4,943,533, Mendelsohn et al.) and variants thereof, such as chimerized 225 (C225) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.).

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

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

The terms "HER2", "ErbB2" "c-Erb-B2" are used interchangeably. Unless indicated otherwise, the terms "ErbB2" "c-Erb-B2" and "HER2" when used herein refer to the human protein, and "erbB2," "c-erb-B2," and "her2" refer to human gene. The human erbB2 gene and ErbB2 protein are, for example, described in Semba et al., *PNAS (USA)* 82:6497–650 (1985)and Yamamoto et al. *Nature* 319:230–234 (1986) (Genebank accession number X03363). ErbB2 comprises four domains (Domains 1–4).

The "epitope 4D5" is the region in the extracellular domain of ErbB2 to which the antibody 4D5 (ATCC CRL 10463) binds. This epitope is close to the transmembrane region of ErbB2. To screen for antibodies which bind to the 4D5 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed (see FIG. **1**) to assess whether the antibody binds to the 4D5 epitope of ErbB2 (i.e. any one or more residues in the region from about residue 529, e.g. about residue 561 to about residue 625, inclusive).

The "epitope 3H4" is the region in the extracellular domain of ErbB2 to which the antibody 3H4 binds. This epitope is shown in FIG. **1**, and includes residues from about 541 to about 599, inclusive, in the amino acid sequence of ErbB2 extracellular domain.

The "epitope 7C2/7F3" is the region at the N-terminus of the extracellular domain of ErbB2 to which the 7C2 and/or 7F3 antibodies (each deposited with the ATCC, see below) bind. To screen for antibodies which bind to the 7C2/7F3 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to establish whether the antibody binds to the 7C2/7F3 epitope on ErbB2 (i.e. any one or more of residues in the region from about residue 22 to about residue 53 of ErbB2; SEQ ID NO:2).

The term "induces cell death" or "capable of inducing cell death" refers to the ability of the antibody to make a viable cell become nonviable. The "cell" here is one which expresses the ErbB2 receptor, especially where the cell overexpresses the ErbB2 receptor. A cell which "overexpresses" ErbB2 has significantly higher than normal ErbB2 levels compared to a noncancerous cell of the same tissue type. Preferably, the cell is a cancer cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SKBR3, BT474, Calu 3, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Cell death in vitro may be determined in the absence of complement and immune effector cells to distinguish cell death induced by antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Thus, the assay for cell death may be performed using heat inactivated serum (i.e. in the absence of complement) and in the absence of immune effector cells. To determine whether the antibody is able to induce cell death, loss of membrane integrity as evaluated by uptake of propidium iodide (PI), trypan blue (see Moore et al. *Cytotechnology* 17:1–11 [1995]) or 7AAD can be assessed relative to untreated cells. Preferred cell death-inducing antibodies are those which induce PI uptake in the "PI uptake assay in BT474 cells".

The phrase "induces apoptosis" or "capable of inducing apoptosis" refers to the ability of the antibody to induce programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is one which overexpresses the ErbB2 receptor. Preferably the "cell" is a tumor cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SKBR3, BT474, Calu 3 cell, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering as disclosed-in the example herein; and nuclear/chromatin condensation-along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an "annexin binding assay using BT474 cells" (see below).

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

ErbB2/ErbB3 receptor complex by HRG (e.g. 7C2). Further, the antibody may be one like 7C2 which, while inducing apoptosis, does not induce a large reduction in the percent of cells in S phase (e.g. one which only induces about 0–10% reduction in the percent of these cells relative to control).

The antibody of interest may be one like 7C2 which binds specifically to human ErbB2 and does not significantly cross-react with other proteins such as those encoded by the 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).

"Heregulin" (HRG) when used herein refers to a polypeptide which activates the ErbB2-ErbB3 and ErbB2-ErbB4 protein complexes (i.e. induces phosphorylation of tyrosine residues in the complex upon binding thereto). Various heregulin polypeptides encompassed by this term are disclosed in Holmes et al., *Science*, 256:1205–1210 (1992); WO 92/20798; Wen et al., *Mol. Cell. Biol.*, 14(3): 1909–1919 (1994); *Nature*, 362:312–318 (1993), for example. The term includes biologically active fragments and/or variants of a naturally occurring HRG polypeptide, such as an EGF-like domain fragment thereof (e.g. $HRG\beta1_{177-244}$)

The "ErbB2-ErbB3 protein complex" and "ErbB2-ErbB4 protein complex" are noncovalently associated oligomers of the ErbB2 receptor and the ErbB3 receptor or ErbB4 receptor, respectively. The complexes form when a cell expressing both of these receptors is exposed to HRG and can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski et al., *J. Biol. Chem.*, 269(20):14661–14665 (1994).

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

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

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the 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 name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

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

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

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

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

The term "antibody" is use d in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal

antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

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

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. 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 inmmunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature*, 321:522–525 (1986); Reichmann et al., *Nature*, 332:323–329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593–596 (1992). The humanized antibody includes a PRIMATIZED™ antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

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

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

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

As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

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

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

A "disorder" is any condition that would benefit from treatment with the anti-ErbB2 antibody. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemias and lymphoid malignancies; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The term "therapeutically effective amount" is used to refer to an amount having antiproliferative effect. Preferably, the therapeutically effective amount has apoptotic activity, or is capable of inducing cell death, and preferably death of benign or malignant tumor cells, in particular cancer cells. 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 cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include 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, 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. I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially an ErbB2-overexpressing cancer cell either in vitro or in vivo. Thus, the growth inhibitory agent is one which significantly reduces the percentage of ErbB2 overexpressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), TAXOL®, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W B Saunders: Philadelphia, 1995), especially p. 13. The 4D5 antibody (and functional equivalents thereof) can also be employed for this purpose.

"Doxorubicin" is an athracycline antibiotic. The full chemical name of doxorubicin is (8S-cis)-10-[(3-amino-2,

3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

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

By "solid phase" is meant a non-aqueous matrix to which the antibodies used in accordance with the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g.,an 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 antibodies disclosed herein and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

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

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 "peak serum concentration" refers to the maximal 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 drug 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, preferable 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 in 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.

Published information related to anti-ErbB2 antibodies includes the following issued patents and published applications: PCT/US89/00051,published Jan. 5,1989; PCT/US90/02697, published May 18,1990; EU 0474727 issued Jul. 23, 1997; DE 69031120.6, issued Jul. 23, 1997; PCT/US97/18385, published Oct. 9 1997; SA 97/9185, issued Oct. 14, 1997; U.S. Pat. No. 5,677,171, issued Oct. 14, 1997; U.S. Pat. No. 5,720,937, issued Feb. 24, 1998; U.S. Pat. No. 5,720,954, issued Feb. 24, 1998; U.S. Pat. No. 5,725,856, issued Mar. 10, 1998; U.S. Pat. No. 5,770,195, issued Jun. 23, 1998; U.S. Pat. No. 5,772,997, issued Jun. 30, 1998; PCT/US98/2626, published Dec. 10, 1998; and PCT/US99/06673, published Mar. 26, 1999, each of which patents and publications is herein incorporated by reference in its entirety.

II. Production of anti-ErbB2 Antibodies

A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention. The ErbB2 antigen to be used for production of antibodies may be, e.g., a soluble form of the extracellular domain of ErbB2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing ErbB2 at their cell surface (e.g. NIH-3T3 cells transformed to overexpress ErbB2; or a carcinoma cell line such as SKBR3 cells, see Stancovski et al., *PNAS (USA)* 88:8691–8695 [1991]) can be used to generate antibodies. Other forms of ErbB2 useful for generating antibodies will be apparent to those skilled in the art.

(i) Polyclonal Antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with ⅕ to ⅒ the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal Antibodies

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

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

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

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

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

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

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

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59–103 [Academic Press, 1986]). Suitable culture media for this purpose include, for example, D-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 imnmunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

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

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

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

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

(iii) Humanized and Human Antibodies

Methods for humanizing non-human antibodies are well known in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import"variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature*, 321:522–525 (1986); Riechmann et al., *Nature*, 332:323–327 (1988); Verhoeyen et al., *Science* 239:1534–1536 [1988]), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some 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. Immnol.*, 151:2623 [1993]).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the

functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits et al., *Nature*, 362:255–258 (1993); Bruggermann et al., *Year in Immuno.*, 7:33 (1993). Human antibodies can also be derived from phage-display libraries (Hoogenboom et al., *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581–597 [1991]).

(iv) Antibody Fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al. , *Journal of Biochemical and Biophysical Methods* 24:107–117(1992) and Brennan et al., *Science*, 229:81 [1985]). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al., *Bio/Technology* 10: 163–167 [1992]). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185.

(v) Bispecific Antibodies

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the ErbB2 protein. For example, one arm may bind an epitope in Domain 1 of ErbB2 such as the 7C2/7F3 epitope, the other may bind a different ErbB2 epitope, e.g. the 4D5 epitope. Other such antibodies may combine an ErbB2 binding site with binding site(s) for EGFR, ErbB3 and/or ErbB4. Alternatively, an anti-ErbB2 arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the ErbB2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express ErbB2. These antibodies possess an ErbB2-binding arm and an arm which binds the cytotoxic agent (e.g. saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

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

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

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

According to another approach described in WO96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target

immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. 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. Shalaby et al., *J. Exp. Med.*, 175: 217–225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

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

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

(vi) Screening for Antibodies With the Desired Properties

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

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

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

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

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

To identify anti-ErbB2 antibodies which inhibit growth of SKBR3 cells in cell culture by 50–100%, the SKBR3 assay described in WO 89/06692 can be performed. According to this assay, SKBR3 cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillinstreptomycin. The SKBR3 cells are plated at 20,000 cells in a 35 mm cell culture dish

(2 mls/35 mm dish) 2.5μg/ml of the anti-ErbB2 antibody is added per dish. After six days, the number of cells, compared to untreated cells are counted using an electronic COULTER™ cell counter. Those antibodies which inhibit growth of the SKBR3 cells by 50–100% are selected for combination with the apoptotic antibodies as desired.

(vii) Effector Function Engineering

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

(viii) Immunoconjugates

The invention also pertains to immunoconjugates comprising the antibody described herein conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g. an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated anti-ErbB2 antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al. *Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 94/11026.

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

(ix) Immunoliposomes

The anti-ErbB2 antibodies disclosed herein may also be formulated as inununoliposomes. 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). S 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 covert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, e.g., Massey, *Nature* 328: 457458 [1987]). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the anti-ErbB2 antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active

portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g., Neuberger et al., *Nature*, 312: 604–608 [1984]).

(xi) Antibody-salvage Receptor Binding Epitope Fusions

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

A systematic method for preparing such an antibody variant having an increased in vivo half-life comprises several steps. The first involves identifying the sequence and conformation of a salvage receptor binding epitope of an Fc region of an IgG molecule. Once this epitope is identified, the sequence of the antibody of interest is modified to include the sequence and conformation of the identified binding epitope. After the sequence is mutated, the antibody variant is tested to see if it has a longer in vivo half-life than that of the original antibody. If the antibody variant does not have a longer in vivo half-life upon testing, its sequence is further altered to include the sequence and conformation of the identified binding epitope. The altered antibody is tested for longer in vivo half-life, and this process is continued until a molecule is obtained that exhibits a longer in vivo half-life.

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

The epitope preferably constitutes a region wherein any one or more amino acid residues from one or two loops of a Fc domain are transferred to an analogous position of the antibody fragment. Even more preferably, three or more residues from one or two loops of the Fc domain are transferred. Still more preferred, the epitope is taken from the CH2 domain of the Fc region (e.g., of an IgG) and transferred to the CH1, CH3, or V_H, region, or more than one such region, of the antibody. Alternatively, the epitope is taken from the CH2 domain of the Fc region and transferred to the C_L region or V_L region, or both, of the antibody fragment.

In one most preferred embodiment, the salvage receptor binding epitope comprises the sequence (5' to 3'): PKNSSMISNTP (SEQ ID NO:3), and optionally further comprises a sequence selected from the group consisting of HQSLGTQ (SEQ ID NO:4), HQNLSDGK (SEQ ID NO:5), HQNISDGK (SEQ ID NO:6), or VISSHLGQ (SEQ ID NO:7), particularly where the antibody fragment is a Fab or $F(ab')_2$. In another most preferred embodiment, the salvage receptor binding epitope is a polypeptide containing the sequence(s) (5' to 3'): HQNLSDGK (SEQ ID NO:5), HQNISDGK (SEQ ID NO:6), or VISSHLGQ (SEQ ID NO:7) and the sequence: PKNSSMISNTP (SEQ ID NO:3).

(xii) 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 filter, for example, an 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 antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite 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 C_H3 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 SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following 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 quantitate the amount of specific rhuMAb HER2 (humanized anti-$p185^{HER2}$ 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-$p185^{HER2}$ 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 $p185^{HER2}$ (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 coatin 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 are washed again with water as in previous steps.

Horse radish peroxidase-conjugate (HRP-conjugate, Goat anti-human IgG Fc 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_2O_2 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 parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; met al complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Preferred lyophilized anti-ErbB2 antibody formulations are described in WO 97/04801, expressly incorporated herein be 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-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

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

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(−)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C., resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying 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 blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The antibodies of the invention are administered to a human patient, in accord with known methods, such as

intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous 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 antracycline) is desired, the combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. 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 μg/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 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. 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 subcutaneous 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-

tions for use, including, e.g., a warning that the composition is not to be used in combination with anthacycline-type chemotherapeutic agent, e.g. doxorubicin or epirubicin.

Deposit of Materials

The following hybridoma cell lines have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md., USA (ATCC):

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

Further details of the invention are illustrated by the following non-limiting Examples.

EXAMPLES

Example 1

Preparation and Efficacy of HERCEPTIN® Anti-ErbB2 Antibody

Materials and Methods

Anti-ErbB2 monoclonal antibody The anti-ErbB2 $IgG_1\kappa$ murine monoclonal antibody 4D5, specific for the extracellular domain of ErbB2, was produced as described in Fendly et al., *Cancer Research* 50: 1550–1558 (1990) and WO89/06692. Briefly, NIH 3T3/HER2–3_{400} cells (expressing approximately 1×10^5 ErbB2 molecules/cell) produced as described in Hudziak et al., *Proc. Natl. Acad. Sci.* (*USA*) 84:7159 (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 ml of the ErbB2 preparation and the splenocytes were fused with mouse myeloma line X63-Ag8.653. Hybridoma supernatants were screened for ErbB2-binding by ELISA and radioimmunoprecipitation. MOPC-21 (IgG1), (Cappell, Durham, N.C.), was used as an isotype-matched control.

The treatment was performed with a humanized version of the murine 4D5 antibody (HERCEPTIN® anti-ErbB2 antibody). The humanized antibody was engineered by inserting the complementarity determining regions of the murine 4D5 antibody into the framework of a consensus human immunoglobulin IgG_1 (IgG_1) (Carter et al., *Proc. Natl. Acad. Sci. USA* 89:4285–4289 [1992]). The resulting humanized anti-ErbB2 monoclonal antibody has high affinity for $p185^{HER2}$ (Dillohiation constant $[K_d]$=0.1 nmol/L), markedly inhibits, in vitro and in human xenografts, the growth of breast cancer cells that contain high levels of p_{185}^{HER2}, induces antibody-dependent cellular cytotoxicity (ADCC), and has been found clinically active, as a single agent, in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior therapy. HERCEPTIN® anti-ErbB2 antibody is produced by a genetically engineered Chinese Hamster Ovary (CHO) cell line, grown in large scale, that secretes the antibody into the culture medium. The antibody is purified from the CHO culture media using standard chromatographic and filtration methods. Each lot of antibody used in this study was assayed to verify identity, purity, and potency, as well as to meet Food and Drug Administration requirements for sterility and safety.

Eligibility Criteria

Patients had to fulfill all of the following criteria to be eligible for study admission:

Metastatic breast cancer

Overexpression of the ErbB2 (HER2) oncogene (2+ to 3+ as determined by immunohistochemistry or fluorescence in situ hybridization (FISH). [Tumor expression of ErbB2 can be determined by immunohistochemical analysis, as previously described (Slamon et al., [1987] and [1989], supra), of a set of thin sections prepared from the patient's paraffim-archived tumor blocks. The primary detecting antibody used is murine 4D5 MAb, which has the same CDRs as the humanized antibody used for the treatment. Tumors are considered to overexpress ErbB2 if at least 25% of tumor cells exhibit characteristic membrane staining for p_{185}^{HER2}].

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:

Prior cytotoxic chemotherapy for metastatic breast cancer

Patients may have received prior hormonal therapy (e.g. tamoxifen) for metastatic disease or cytotoxic therapy in the adjuvant setting.

Concomitant malignancy that has not been curatively treated

A performance status of <60% on the Karnofsky scale

Pregnant or nursing women; women of childbearing potential, unless using effective contraception as determined by the investigator

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)

Use of investigational or unlicensed agents within 30 days prior to study entry

Clinically unstable or untreated metastases to the brain (e.g. requiring radiation therapy)

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

Anti-ErbB2 Antibody

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 (T, 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/m^2) 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/m^2) or epirubicin (75 mg/m^2) 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.

Paciltaxel (TAXOL®) was given at a dose of 175 mg/m^2 over 3 hours by intravenous administration. All patients receiving paclitaxel were premedicated with dexamethasone (or its equivalent) 20 mg×2, administered orally 12 and 6 hours prior to paclitaxel; diphenhydramine (or its equivalent) 50 mg, iv, administered 30 minutes prior to paclitaxel, and dimetidine (or another H_2 blocker) 300 mg, iv, administered 30 minutes prior to paclitaxel.

Response Criteria

Progressive Disease

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 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 1

HERCEPTIN ® Anti-ErbB2 Antibody Efficacy

	Enrolled	TTP (months)	RR (%)	AE (%)
CRx	234	5.5	36.2	66
CRx + 14	235	8.6*	62.00**	69
AC	145	6.5	42.1	71
AC + H	146	9.0	64.9	68
T	89	4.2	25.0	59
T + H	89	7.1	57.3	70

*$p < 0.001$ by log-rank test; ** $p < 0.01$ by X^2 test; CRx: chemotherapy; AC: anthracycline/cyclophosphamide treatment; 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 ¾) 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.

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

HERCEPTIN ® Anti-ErbB2 Antibody Trough and Peak Serum Concentrations for the First 8 Weeks of Treatment (μg/ml)

	Dose Number	n	Mean	SD	Minimum	Maximum
Peak	1	195	100.3	35.2	30.7	274.6
Trough		195	25.0	12.7	0.16	60.7
Peak	2	190	74.3	31.3	20.8	307.9
Trough		167	30.4	16.0	0.2	74.4
Peak	3	167	75.3	26.8	16.1	194.8
Trough		179	33.7	17.9	0.2	98.2
Peak	4	175	80.2	26.9	22.2	167
Trough		132	38.6	20.1	0.2	89.4
Peak	5	128	85.9	29.2	27.8	185.8
Trough		141	42.1	24.8	0.2	148.7
Peak	6	137	87.2	32.2	28.9	218.1
Trough		115	43.2	24.0	0.2	109.9
Peak	7	114	89.7	32.5	16.3	187.8
Trough		137	48.8	24.9	0.2	105.2
Peak	8	133	95.6	35.9	11.4	295.6

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 (n=40, 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=12.6). 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 overexpresses 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 3×10^6 BT474M 1 cells suspended in Matrigel™. When tumor nodules reached a volume of approximately 100 mm^3, animals were randomized to 4 treatment groups. The groups were treated according to Table 3.

TABLE 3

Animal Groups and Doses for Comparison of I.V. Bolus and S.C. Infusion

Group, Dose, Antibody	Target Serum Conc. μg/ml	Route of Administration	Loading Dose (mg/kg)	Maintenance Dose
1-Control, rhuMAb E25	20	IV LD and SC infusion	2.20	0.250 mg/ml (infusate)
2-Low Dose SC rhuMAb HER2	1	IV LD and SC infusion	0.313	0.050 mg/ml (infusate)
3-High Dose SC rhuMAb HER2	20	IV LD and SC infusion	6.25	1.00 mg/ml (infusate)
4-IV Multi-Dose rhuMAb HER2	20 (trough)	IV LD and MD	4.00	2 mg/kg/week (IV bolus)

Serum Conc. = concentration in serum.
LD = loading dose.
MD = maintenance dose.
Infusate concentration was calculated to achieve targeted serum concentration using Alzet ® osmotic minipumps (Alza Corp., Palo Alto, CA).

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 nonrelevant antibody E25 (non-specific for HER2 receptor, but a member of the monoclonal IgG class) or test antibody HERCEPTIN® anti-ErbB2 anitbody 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 6 to day 35 in the study for which data is tabulated below. Tumors were measured in three dimensions and volumes were expressed in mm^3. 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

Comparison of S.C. Infusion and I.V. Bolus Delivery

Treatment Group	Tumor Volume (mm^3), Day 35, (n = 14)	Tumor Volume (area under curve) Day 6–Day 35 (n = 13)	HERCEPT ® Serum Conc. (μg/ml), Day 27, (n = 3)
control s.c. infusion	764 ± 700	5650 ± 4700	4.16 ± 1.94
s.c. infusion (low dose)	80.6 ± 158	1610 ± 1250	2.11 ± 1.74
s.c. infusion (high dose)	31 ± 75.6	1440 ± 1140	22.1 ± 5.43
i.v. bolus dose*	49.7 ± 95.7	2150 ± 1480	21.7 ± 17.1**

s.c. = subcutaneous delivery; i.v. = intravenous delivery.
*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 innoculation as described in Example 3. Six days after tumor cell innoculation, the initial tumor measurement was performed. Seven days after tumor cell innoculation, 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

Animal Groups and Doses for Comparison of I.V. Bolus and S.C Bolus Delivery

Group	Route of Administration	Loading Dose (mg/kg)	Maintenance Dose (mg/kg/week)	n
1-Control rbuMAb E25	IV	8	4	10
2-rhuMAb HER2	IV	2	1	10
3-rhuMAb HER2	IV	4	2	10
4-rhuMAb HER2	IV	8	4	10
5-rhuMAb HER2	SC	4	2	10

IV = intraveneous; 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

IV versus SC Bolus Delivery:
Serum HERCEPTIN ® Anti-ErbB2 Antibody Concentration
Serum Concentration, μg/ml

Treatment Group (delivery, MD)	Day 0 Mean (SD)	Day 7 Mean (SD)	Day 14 Mean (SD)	Day 21 Mean (SD)
1-Control rhu MAb E25	0	25.9	34.6	38.5
(IV, 4mg/kg)	(0)	(8.29)	(11.2)	(14.4)
2-rhu MAb HER2	0	4.96	8.55	8.05
(IV, 1 mg/kg)	(0)	(3.79)	(5.83)	(4.67)
3-rhu MAb HER2	0	13.4	18.9	22.6
(IV, 2 mg/kg)	(0)	(9.24)	(12.0)	(9.21)
4-rhu MAb HER2	0	29.6	37.7	46.2
(IV, 4 mg/kg)	(0)	(13.5)	(14.4)	(13.8)
5-rhu MAb HER2	0	12.5	16.9	17.6
(SC, 2 mg/kg)	(0)	(7.33)	(10.2)	(10.7)

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.

TABLE 7

Efficacy of HERCEPTIN ® Anti-ErbB2 Antibody Measured as a Change in Tumor Volume Comparing Intravenous Bolus and Subcutaneous Bolus Delivery, Mean (SD)

Treatment Group (Delivery, MD)	Tumor Vol. Day 6, mm^3	Tumor Vol. Day 28 mm^3	Tumor Vol. Day 31, mm^3	Day 6–Day 31* Area Under Curve Tumor Vol., mm^3	Tumor Growth Rate on Log (TM + 1)
1-IV Control	321	1530	1630	13600	0.0660
	(190)	(1040)	(1170)	(7230)	(0.0200)
2-IV Herceptin	297	175	151	4690	–0.0505
1 mg/kg	(130)	(215)	(188)	(1400)	(0.142)
3-IV Herceptin	269	75.7	73.6	3510	–0.0608
2 mg/kg	(129)	(92.4)	(84.5)	(1220)	(0.110)
4-IV Herceptin	272	25.3	25.8	2880	–0.0810
4 mg/kg	(117)	(75.9)	(72.9)	(1230)	(0.0859)
5-SC Herceptin	268	76.2	90.4	3230	–0.0304
2 mg/kg	(117)	(98.8)	(105)	(1440)	(0.104)

N = 10 for each data point.
TM = tumor measurement.
IV = intravenous.
SC = subcutaneous.
MD = maintenance dose.
Tumor Vol. = tumor volume, mm^3.
*Day 17 excluded due to measurement error.
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.

FIGS. **4**A and **4**B are graphical plots of changes in tumor volume over time, some of which data is found in Table 7. FIG. **4**A is a linear plot of tumor volume versus time. FIG. **4**B is a semilogarithmic plot of the same data, allowing the test points be viewed more clearly. The data in Table 7 and FIGS. **4**A and **4**B indicate that, although a dose-related response was not observed between HERCEPTIN-treated groups, dosing by subcutaneous bolus was as effective as intravenous bolus dosing and achieved similar trough serum concentrations.

Example 5

Regimens for Intravenous and Subcutaneous Delivery of Anti-ErbB2 Antibody

According to the invention, methods of anti-ErbB2 antibody (e.g., HERCEPTIN®) delivery comprise greater front loading of the drug to achieve a 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 of equal or smaller amount. An advantage of the methods of the invention is that the maintenance dosing may be less frequent and/or delivered by subcutaneous injection, making the treatment regimens of the invention convenient and cost-effective for the patient and medical professionals administering the antibody. In addition, a subcutaneous maintenance dose regimen may be interrupted by intravenous dosing (such as infusion) when the patient's chemotherapy requires delivery of other drugs by intravenous injection.

To test the following dosage regimens, human subjects are selected according to the criteria disclosed in Example 1, above. The number of initial doses is one or more doses 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 1 day or less. The number of maintenance doses may be one or more doses sufficient to achieve suppression of disease symptoms, such as a decrease in tumor volume. The maintenance doses are 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 delivery subsequent maintenance doses.

In another method, an initial (front loading) dose of 8 mg/kg HERCEPTIN® anti-ErbB2 antibody is delivered by intravenous injection (infusion 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 injection (infusion 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 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 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 example, 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® 0 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 is optionally and preferably deliverable 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 anthrocycline 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/m^2 every 3 weeks) or an anthracycline derivative (e.g. doxorubicin 60 mg/m^2 or epirubicin 75 mg/m^2 every 3 weeks). Optionally, where an anthracycline derivative is administered, a cardioprotectant (e.g. 600 mg/m^2 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/m^2/week. According to another embodiment of the invention, the taxoid is docetaxel and is administered at a dose of 30–70 mg/m^2/week.

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/m^2 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 spermicidal 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 3.0×10^9/l
   Granulocytes less than 1.5×109/l
   Platelets less than 100×109/l
8) Any of the following abnormal baseline liver function tests:
   Serum bilirubin greater than 1.5× ULN (upper normal limit)
   ALT and/or AST greater than 2.5× ULN (greater than 4.0× ULN if liver or bone metastasis)
   Alkaline phosphatase greater than 2.5× ULN (greater than 4.0× ULN if liver or bone metastasis)
9) The following abnormal baseline renal function tests: serum creatinine greater than 1.5× 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/m^2 IV every 3 weeks×6 cycles as a 3-hour infusion. NOTE: On the first cycle of treatment,

paclitaxel will be dosed 8 hours prior to HERCEPTIN® to determine the PK of paclitaxel alone. HERCEPTIN® will be administered 8 hours post-paclitaxel for the 1st cycle only. In subsequent treatment cycles, HERCEPTIN® will be administered prior to paclitaxel.

The total duration of this study is 18 weeks. Study subjects will receive up to 6 total HERCEPTIN® doses. After the last subject has received the last cycle of paclitaxel, data collection for safety and pharmacokinetic analysis will stop, and the study will close to protocol specified treatment. Study subjects may continue to receive the HERCEPTIN® +/− paclitaxel at the discretion of the investigator.

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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Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Val Gly Tyr Ser Leu
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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 two 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.

* * * * *

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

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(45) **Date of Patent: May 13, 2008**

(54) **DOSAGES FOR TREATMENT WITH ANTI-ERBB2 ANTIBODIES**

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(57) **ABSTRACT**

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.

40 Claims, 5 Drawing Sheets

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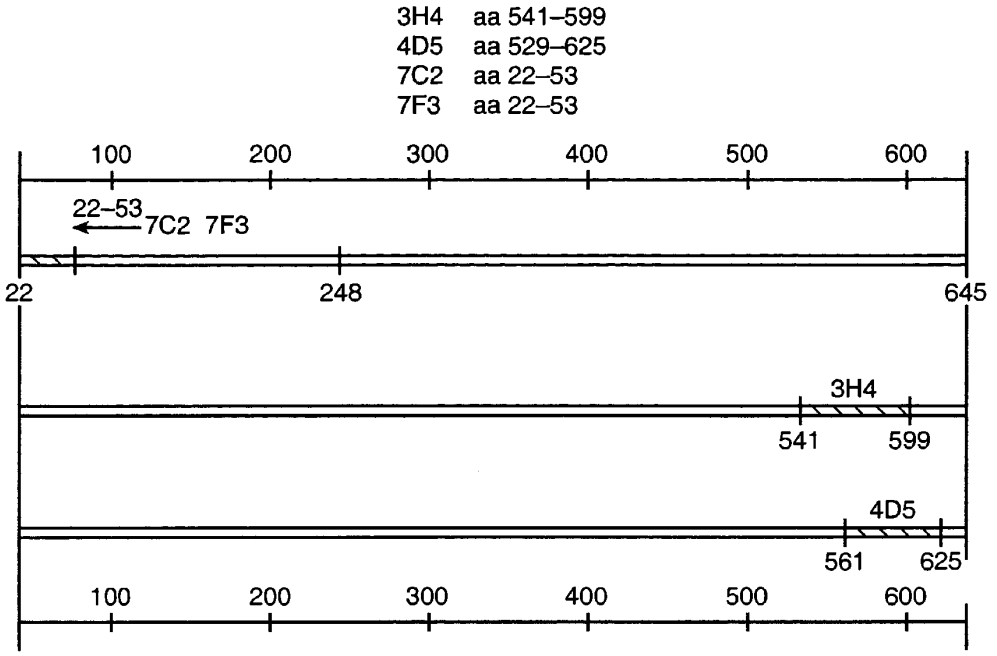

3H4 epitope (SEQ ID NO:8) 58 residues

VEECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVAR

541 **599**

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

LPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQP

561 **625**

FIG._1

1 MELAALCRWGLLLALLPPGAA**STQVCTGTDMKLRLPA**

38 **SPETHLDMLRHLYQGC**QVVQGNLELTYLPTNASLSFL

75 QDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDN

112 YALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEI

149 LKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLID

186 TNRSRA

FIG._2

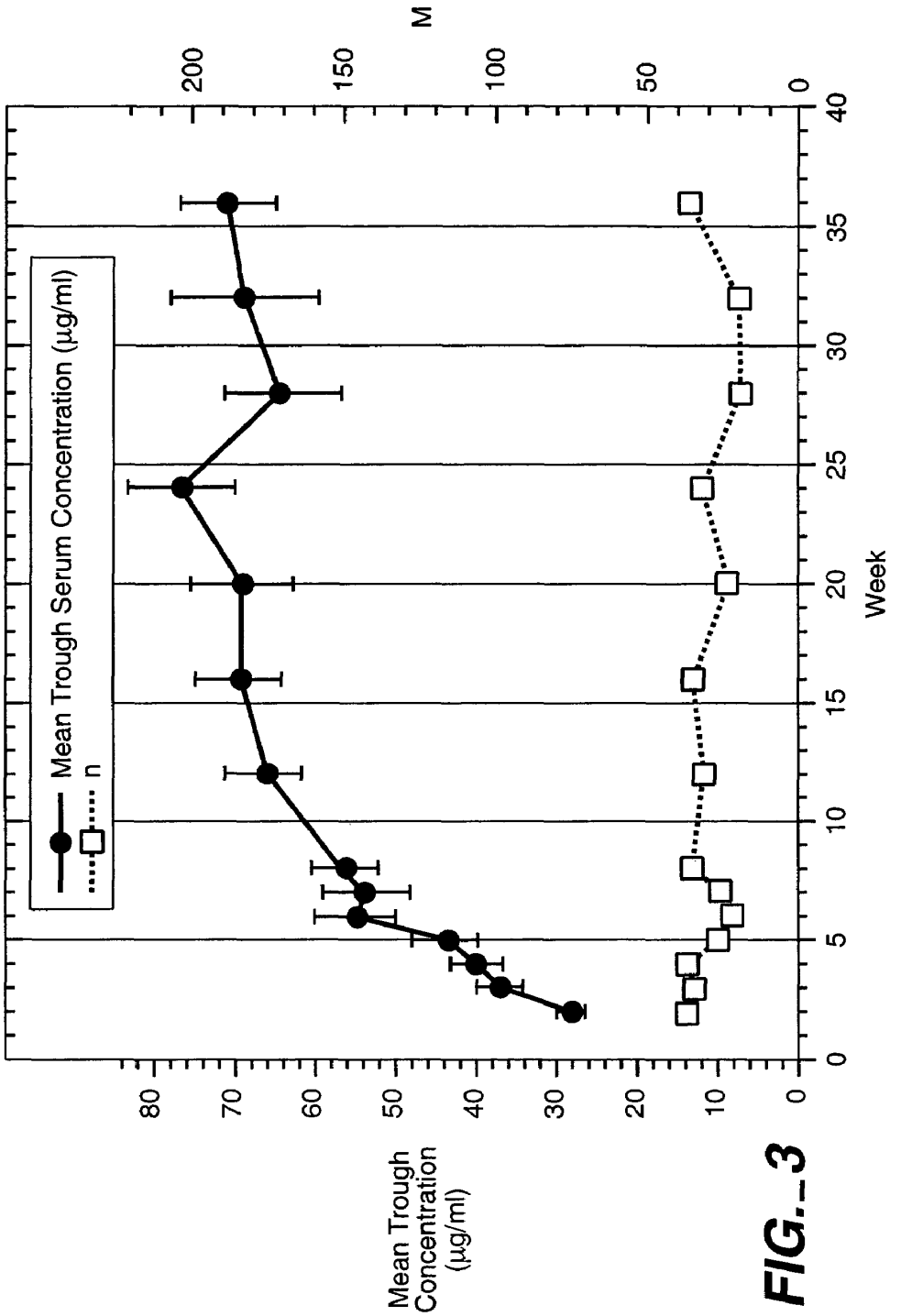
Mean Trough Serum Concentration (µg/ml)
n
Mean Trough Concentration (µg/ml)
Week
M
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25
30
35
40
0
50
100
150
200

FIG._3

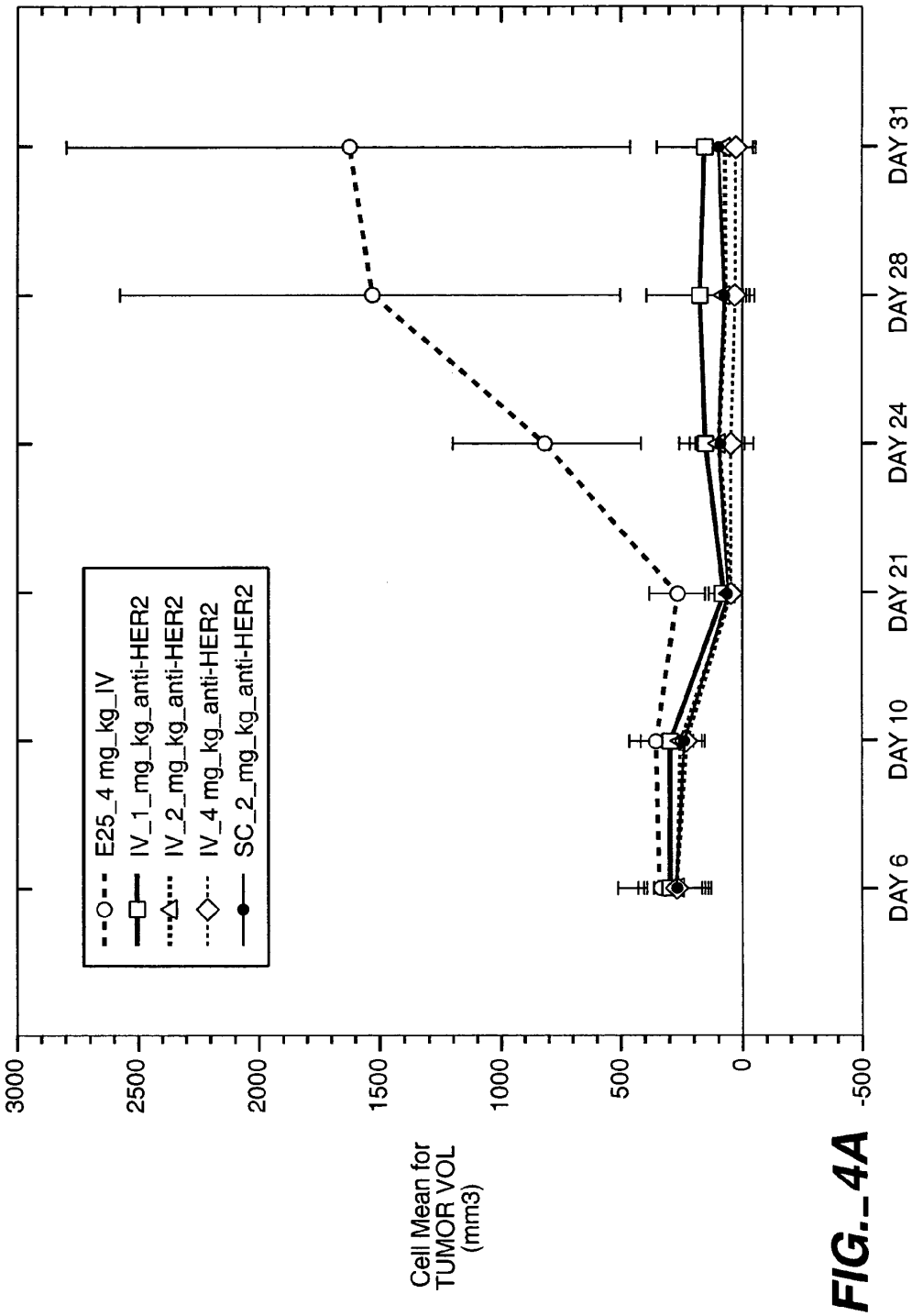
E25_4 mg_kg_IV
IV_1_mg_kg_anti-HER2
IV_2_mg_kg_anti-HER2
IV_4 mg_kg_anti-HER2
SC_2_mg_kg_anti-HER2
3000
2500
2000
1500
1000
500
0
-500
DAY 6
DAY 10
DAY 21
DAY 24
DAY 28
DAY 31
Cell Mean for TUMOR VOL (mm3)

FIG._4A

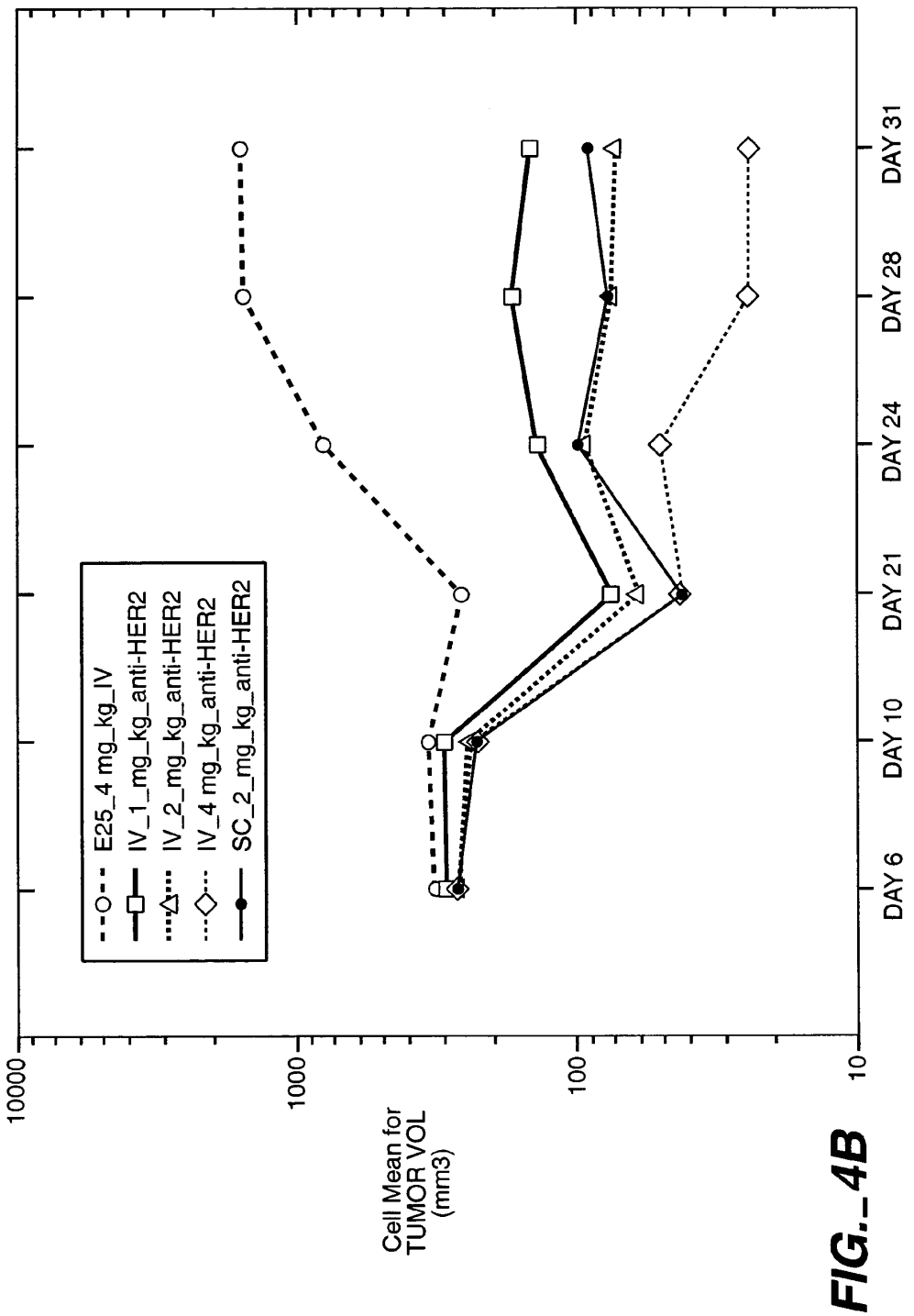
E25_4 mg_kg_IV
IV_1_mg_kg_anti-HER2
IV_2_mg_kg_anti-HER2
IV_4 mg_kg_anti-HER2
SC_2_mg_kg_anti-HER2
10000
1000
100
10
Cell Mean for TUMOR VOL (mm3)
DAY 6
DAY 10
DAY 21
DAY 24
DAY 28
DAY 31

FIG._4B

VARIABLE LIGHT

```
          1       10        20          30              40
2C4       DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIGVA----] WYQQRP
           **    **** *      *                         *
574       DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA----] WYQQKP
                                   *   *  ****
hum kI    DIQMTQSPSSLSASVGDRVTITC [RASQSVSTSSYSYMH] WYQQKP

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

                   90          100
2C4       EDLAVYYC [QQYYIYPYT] FGGGTKLEIK (SEQ ID NO:10)
            * *                 *   *
574       EDFATYYC [QQYYIYPYT] FGQGTKVEIK (SEQ ID NO:12)
                      ***
hum kI    EDFATYYC [QQYNSLPYT] FGQGTKVEIK (SEQ ID NO:14)
```

FIG._5A

VARIABLE HEAVY

```
          1       10        20          30          40
2C4       EVQLQQSGPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS
              **  **  *  * ***  **                  *
574       EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA
                                       ** * *
humIII    EVQLVESGGGSVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                    50        60          70        80
2C4       HGKSLEWIG [DVNPNSGGSIYNQRFKG] KASLTVDRSSRIVYM
          *  *   **                     *** *    **** *
574       PGKGLEWVA [DVNPNSGGSIYNQRFKG] RFTLSVDRSKNTLYL
                 *  ******  ** ****       *. * *
humIII    PGKGLEWVS [VISGDGGSTYYADSVKG] RFTISRDDSKNTLYL

                     90         100           110
2C4       ELRSLTFEDTAVYYCAR [NLGPSFYFDY] WGQGTTLVTSS (SEQ ID NO:11)
          ***  **                              *
574       QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS (SEQ ID NO:13)
                            ** ***
humIII    QMNSLRAEDTAVYYCAR [GRGGGS--DY] WGQGTLVTVSS (SEQ ID NO:15)
```

FIG._5B

DOSAGES FOR TREATMENT WITH ANTI-ERBB2 ANTIBODIES

RELATED APPLICATIONS

This application is divisional of U.S. Ser. No. 09/648,067 filed Aug. 25, 2000 (now U.S. Pat. No. 6,627,196), which claims priority under 35 USC 119(e) to provisional application Nos. 60/151,018, filed Aug. 27, 1999 and 60/213,822, filed Jun. 23, 2000, the contents of which are incorporated heroin 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 ($p185^{HER2}$) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer (Slamon et al., *Science* 235:177-182 [1987]; Slamon et al., *Science* 244:707-712 [1989]).

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

Antibodies directed against human erbB2 protein products and proteins encoded by the rat equivalent of the erbB2 gene (neu) have been described. Drebin et al., *Cell* 41:695-706 (1985) refer to an IgG2a monoclonal antibody which is directed against the rat neu gene product. This antibody called 7.16.4 causes down-modulation of cell surface p185 expression on B104-1-1 cells (NIH-3T3 cells transfected with the neu proto-oncogene) a inhibits colony formation of these cells. In Drebin et al. *PNAS* (*USA*) 83:9129-9133 (1986), the 7.16.4 antibody was shown to inhibit the tumorigenic growth of neu-transformed NIH-3T3 cells as well as rat neuroblastoma cells (from which the neu oncogene was initially isolated) implanted into nude mice. Drebin et al. in *Oncogene* 2:387-394 (1988) discuss the production of a panel of antibodies against the rat neu gene product. Al of the antibodies were found to exert a cytostatic effect on the growth of neu-transformed cells suspended in soft agar. Antibodies of the IgM, IgG2a and IgG2b isotypes were able to mediate significant in vitro lysis of neu-transformed cells in the presence of complement, whereas none of the antibodies were able to mediate high levels of antibody-dependent cellular cytotoxicity (ADCC) of the neu-transformed cells. Drebin et al. *Oncogene* 2:273-277 (1988) report that mixtures of antibodies reactive with two distinct regions on the p185 molecule result in synergistic anti-tumor effects on neu-transformed NIH-3T3 cells implanted into nude mice. Biological effects of anti-neu antibodies are reviewed in Myers et al., *Meth. Enzym.* 198:277-290 (1991). See also WO94/22478 published Oct. 13, 1994. Hudziak et al., *Mol. Cell. Biol.* 9(3): 1165-1172 (1989) describe the generation of a panel of anti-ErbB2 antibodies which were characterized using the human breast tumor cell line SKBR3. Relative cell proliferation of the SKBR3 cells following exposure to the antibodies was determined by crystal violet staining of the monolayers after 72 hours. Using this assay, maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel, including 7C2 and 7F3, reduced cellular proliferation to a lesser extent in this assay. Hudziak et al. conclude that the effect of the 4D5 antibody on SKBR3 cells was cytostatic rather than cytotoxic, since SKBR3 cells resumed growth at a nearly normal rate following removal of the antibody from the medium. The antibody 4D5 was further found to sensitize $p185^{erbB2}$-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also WO89/06692 published 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); Vitetta et al. *Cancer Research* 54:5301-5309 (1994); Sliwkowski et al. *J. Biol. Chem.* 269(20):14661-14665 (1994); Scott et al. *J. Biol. Chem.* 266:14300-5 (1991); and D'souza et al. *Proc. Natl. Acad. Sci.* 91:7202-7206 (1994).

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

McKenzie et al. *Oncogene* 4:543-548 (1989) generated a panel of anti-ErbB2 antibodies with varying epitope specificities, including the antibody designated TA1. This TA1 antibody was found to induce accelerated endocytosis of ErbB2 (see Maier et al. *Cancer Res.* 51:5361-5369 [1991]). Bacus et al. *Molecular Carcinogenesis* 3:350-362 (1990) reported that the TA1 antibody induced maturation of the breast cancer cell lines AU-565 (which overexpresses the 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 fibroblasts 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 fibroblasts overexpressing human ErbB2 was assessed. As observed in their earlier work, N28 accelerated tumor growth, whereas N12 and N29 significantly inhibited growth of the ErbB2-expressing cells. Partial tumor inhibition was also observed with the N24 antibody. Bacus et al. also tested the ability of the antibodies to promote a mature phenotype in the human breast cancer cell lines AU-565 and MDA-MB453 (which overexpress ErbB2) as well as MCF-7 (containing low levels of the receptor). Bacus et al. saw a correlation between tumor inhibition in vivo and cellular differentiation; the tumor-stimulatory antibody N28 had no effect on differentiation, and the tumor inhibitory action of the N12, N29 and N24 antibodies correlated with the extent of differentiation they induced.

Xu et al. *Int. J. Cancer* 53:401-408 (1993) evaluated a panel of anti-ErbB2 antibodies for their epitope binding specificities, as well as their ability to inhibit anchorage-independent and anchorage-dependent growth of SKBR3 cells (by individual antibodies and in combinations), modulate cell-surface ErbB2, and inhibit ligand stimulated anchorage-independent growth. See also WO94/00136 published Jan. 6, 1994 and Kasprzyk et al. *Cancer Research* 52:2771-2776 (1992) concerning anti-ErbB2 antibody combinations. Other anti-ErbB2 antibodies are discussed in Hancock et al. *Cancer Res.* 51:4575-4580 (1991); Shawver et al. *Cancer Res.* 54:1367-1373 (1994); Arteaga et al. *Cancer Res.* 54:3758-3765 (1994); and Harwerth et al. *J. Biol. Chem.* 267:15160-15167 (1992).

A recombinant humanized anti-ErbB2 monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2, 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 (Baselga 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 Chamness, *Gene* 159:19-27 [1995]; and Hynes and Stern, *Biochim Biophys Acta* 1198:165-184 [1994]), and has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluoruracil) and anthracyclines (Baselga et al., *Oncology* 11(3 Supp11):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 μg/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 8 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 about 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. Generally, two or more subsequent doses (e.g. from about two to about ten subsequent doses) of the antibody are administered to the patient, and those subsequent doses are preferably 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). The two or more subsequent doses may each be from about 2 mg/kg to about 16 mg/kg; or from about 4 mg/kg to about 12 mg/kg; or from about 6 mg/kg to about 12 mg/kg. 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.

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. The invention additionally provides an article of manufacture, comprising a container, a composition within the container comprising an anti-ErbB 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 label on or associated with the container that indicates that the composition can be used for treating a condition characterized by overexpression of ErbB2 receptor, and a package insert containing instructions to avoid the use of anthracycline-type chemotherapeutics in combination with the composition. According to the invention, the 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 including, but not limited to, paclitaxel and docetaxel. In another embodiment, the chemotherapeutic agent is an anthracyline derivative including, but not limited to, doxorubicin or epirubicin. In still another embodiment of the invention, treatment with an anti-ErbB2 antibody and an anthracycline derivative further includes administration of 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 composition and a chemotherapeutic agent to a patient. In another embodiment, the chemotherapeutic agent is other than an anthracycline, and is preferably a taxoid, such as TAXOL®. In still another embodiment, the chemotherapeutic agent is an anthracycline, including but not limited to, doxorubicin or epirubicin. In yet another embodiment, the chemotherapeutic agent is an anthracycline and the package insert further instructs the user to administer a cardioprotectant.

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 huMAb4D5-8 (HERCEPTIN® anti-ErbB2 antibody); or monoclonal antibody 2C4, e.g., humanized 2C4. The antibody may be an intact antibody (e.g., an intact IgG_1 antibody) or an antibody fragment (e.g., a Fab, $F(ab')_2$, diabody, and the like). The variable light chain and variable heavy chain regions of humanized anti-ErbB2 antibody 2C4 are shown in FIGS. **5**A and **5**B.

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.* 125(6):1395-1406 [June 1994]). The anti-proliferative Mabs 4D5 and 3H4 bind adjacent to the transmembrane domain. The various ErbB2-ECD truncations or point mutations were prepared from cDNA using polymerase chain reaction technology. The ErbB2 mutants were expressed as gD fusion proteins in a mammalian expression plasmid. This expression plasmid uses the cytomegalovirus promoter/enhancer with SV40 termination and polyadenylation signals located downstream of the inserted cDNA. Plasmid DNA was transfected into 293S cells. One day following transfection, the cells were metabolically labeled overnight in methionine and cysteine-free, low glucose DMEM containing 1% dialyzed fetal bovine serum and 25 μCi each of ^{35}S methionine and ^{35}S cysteine. Supernatants were harvested either the ErbB2 MAbs or control antibodies were added to the supernatant and incubated 24 hours at 4° C. The complexes were precipitated, applied to a 10-20% Tricine SDS gradient gel and electrophoresed at 100 V. The gel was electroblotted onto a membrane and analyzed by autoradiography. SEQ ID NOs:8 and 9 depict the 3H4 and 4D5 epitopes, respectively.

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

FIG. **3** is a graph of anti-ErbB2 antibody (HERCEPTIN®) trough serum concentration (μg/ml, mean±SE, dark circles) by week from week 2 through week 36 for ErbB2 overexpressing patients treated with HERCEPTIN® anti-ErbB2 antibody at 4 mg/kg initial dose, followed by 2 mg/kg weekly. The number of patients at each time point is represented by "n" (white squares).

FIG. **4**A is a linear plot of tumor volume changes over time in mice treated with HERCEPTIN® anti-ErbB2 antibody. FIG. **4**B is a semi-logarithmic plot of the same data as in FIG. **4**A such that the variation in tumor volume for the treated animals is observed more readily.

FIGS. **5**A and **5**B depict alignments of the amino acid sequences of the variable light (V_L) (FIG. **5**A) and variable heavy (V_H) (FIG. **5**B) domains of murine monoclonal antibody 2C4 (SEQ ID Nos. 10 and 11, respectively); V_L and V_H domains of humanized Fab version 574 (SEQ ID Nos. 12 and 13, respectively), and human V_L and V_H consensus frameworks (hum κ1, light kappa subgroup I; humIII, heavy subgroup III) (SEQ ID Nos. 14 and 15, respectively). Asterisks identify differences between humanized Fab version 574 and murine monoclonal antibody 2C4 or between humanized Fab version 574 and the human framework. Complementarity Determining Regions (CDRs) are in brackets. Humanized Fab version 574, with the changes ArgH71Val, AspH73Arg and IleH69Leu, appears to have binding restored to that of the original chimeric 2C4 Fab fragment. Additional FR and/or CDR residues, such as L2, L54, L55, L56, H35 and/or H48, may be modified (e.g. substituted as follows—IleL2Thr; ArgL54Leu; TyrL55Glu;

ThrL56Ser; AspH35Ser; and ValH48Ile) in order to further refine or enhance binding of the humanized antibody. Alternatively, or additionally, the humanized antibody may be affinity matured in order to further improve or refine its affinity and/or other biological activities.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

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. *Ann. Rev. Biochem.* 56:881-914 (1987), including variants thereof (e.g. a deletion mutant EGFR as in Humphrey et al. *PNAS (USA)* 87:4207-4211 (1990)). erbB1 refers to the gene encoding the EGFR protein product. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, U.S. Pat. No. 4,943,533, Mendelsohn et al.) and variants thereof, such as chimerized 225 (C225) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.).

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

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

The terms "HER2", "ErbB2" "c-Erb-B2" are used interchangeably. Unless indicated otherwise, the terms "ErbB2" "c-Erb-B2" and "HER2" when used herein refer to the human protein, and "erbB2," "c-erb-B2," and "her2" refer to human gene. The human erbB2 gene and ErbB2 protein are, for example, described in Semba et al., *PNAS (USA)* 82:6497-6501 (1985) and Yamamoto et al. *Nature* 319:230-234 (1986) (Genebank accession number X03363). ErbB2 comprises four domains (Domains 1-4).

The "epitope 4D5" is the region in the extracellular domain of ErbB2 to which the antibody 4D5 (ATCC CRL 10463) binds. This epitope is close to the transmembrane region of ErbB2. To screen for antibodies which bind to the 4D5 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed (see FIG. **1**) to assess whether the antibody binds to the 4D5 epitope of ErbB2 (i.e. any one or more residues in the region from about residue 529, e.g. about residue 561 to about residue 625, inclusive).

The "epitope 3H4" is the region in the extracellular domain of ErbB2 to which the antibody 3H4 binds. This epitope is shown in FIG. **1**, and includes residues from about 541 to about 599, inclusive, in the amino acid sequence of ErbB2 extracellular domain.

The "epitope 7C2/7F3" is the region at the N-terminus of the extracellular domain of ErbB2 to which the 7C2 and/or 7F3 antibodies (each deposited with the ATCC, see below) bind. To screen for antibodies which bind to the 7C2/7F3 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, E d Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to establish whether the antibody binds to the 7C2/7F3 epitope on ErbB2 (i.e. any one or more of residues in the region from about residue 22 to about residue 53 of ErbB2; SEQ ID NO: 2).

The term "induces cell death" or "capable of inducing cell death" refers to the ability of the antibody to make a viable cell become nonviable. The "cell" here is one which expresses the ErbB2 receptor, especially where the cell overexpresses the ErbB2 receptor. A cell which "overexpresses" ErbB2 has significantly higher than normal ErbB2 levels compared to a noncancerous cell of the same tissue type. Preferably, the cell is a cancer cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SKBR3, BT474, Calu 3, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Cell death in vitro may be determined in the absence of complement and immune effector cells to distinguish cell death induced by antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Thus, the assay for cell death may be performed using heat inactivated serum (i.e. in the absence of complement) and in the absence of immune effector cells. To determine whether the antibody is able to induce cell death, loss of membrane integrity as evaluated by uptake of propidium iodide (PI), trypan blue (see Moore et al. *Cytotechnology* 17:1-11 [1995]) or 7AAD can be assessed relative to untreated cells. Preferred cell death-inducing antibodies are those which induce PI uptake in the "PI uptake assay in BT474 cells".

The phrase "induces apoptosis" or "capable of inducing apoptosis" refers to the ability of the antibody to induce programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is one which overexpresses the ErbB2 receptor. Preferably the "cell" is a tumor cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SKBR3, BT474, Calu 3 cell, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering as disclosed in the example herein; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5

to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an "annexin binding assay using BT474 cells" (see below).

Sometimes the pro-apoptotic antibody will be one which blocks HRG binding/activation of the ErbB2/ErbB3 complex (e.g. 7F3 antibody). In other situations, the antibody is one which does not significantly block activation of the ErbB2/ErbB3 receptor complex by HRG (e.g. 7C2). Further, the antibody may be one like 7C2 which, while inducing apoptosis, does not induce a large reduction in the percent of cells in S phase (e.g. one which only induces about 0-10% reduction in the percent of these cells relative to control).

The antibody of interest may be one like 7C2 which binds specifically to human ErbB2 and does not significantly cross-react with other proteins such as those encoded by the 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).

"Heregulin" (HRG) when used herein refers to a polypeptide which activates the ErbB2-ErbB3 and ErbB2-ErbB4 protein complexes (i.e. induces phosphorylation of tyrosine residues in the complex upon binding thereto). Various heregulin polypeptides encompassed by this term are disclosed in Holmes et al., *Science,* 256:1205-1210 (1992); WO 92/20798; Wen et al., *Mol. Cell. Biol.,* 14(3):1909-1919 (1994); and Marchionni et al., *Nature,* 362:312-318 (1993), for example. The term includes biologically active fragments and/or variants of a naturally occurring HRG polypeptide, such as an EGF-like domain fragment thereof (e.g. HRG$\beta 1_{177-244}$).

The "ErbB2-ErbB3 protein complex" and "ErbB2-ErbB4 protein complex" are noncovalently associated oligomers of the ErbB2 receptor and the ErbB3 receptor or ErbB4 receptor, respectively. The complexes form when a cell expressing both of these receptors is exposed to HRG and can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski et al., *J. Biol. Chem.,* 269(20):14661-14665 (1994).

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

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

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the 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. 1, pages 647-669 [1991]). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular 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 name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

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

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

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

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

respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

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

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

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., *Nature,* 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. 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 a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; 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 optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature,* 321:522-525 (1986); Reichmann et al., *Nature,* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.,* 2:593-596 (1992). The humanized antibody includes a PRIMATIZED™ antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

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

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

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

As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of

treatment include those already with the disorder as well as those in which the disorder is to be prevented.

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

A "disorder" is any condition that would benefit from treatment with the anti-ErbB2 antibody. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemias and lymphoid malignancies; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The term "therapeutically effective amount" is used to refer to an amount having antiproliferative effect. Preferably, the therapeutically effective amount has apoptotic activity, or is capable of inducing cell death, and preferably death of benign or malignant tumor cells, in particular cancer cells. 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 cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include 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, 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. I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidaniine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially an ErbB2-overexpressing cancer cell either in vitro or in vivo. Thus, the growth inhibitory agent is one which significantly reduces the percentage of ErbB2 overexpressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), TAXOL®, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The *Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W B Saunders:

Philadelphia, 1995), especially p. 13. The 4D5 antibody (and functional equivalents thereof) can also be employed for this purpose.

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

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

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

By "solid phase" is meant a non-aqueous matrix to which the antibodies used in accordance with the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g.,an 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 antibodies disclosed herein and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

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

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 "peak serum concentration" refers to the maximal 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 drug 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, preferable 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 in 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.

Published information related to anti-ErbB2 antibodies includes the following issued patents and published applications: PCT/US89/0005 1, published Jan. 5, 1989; PCT/US90/02697, published May 18, 1990; EU 0474727 issued Jul. 23, 1997; DE 69031120.6, issued Jul. 23, 1997; PCT/US97/18385, published Oct. 9, 1997; SA 97/9185, issued Oct. 14, 1997; U.S. Pat. No. 5,677,171, issued Oct. 14, 1997; U.S. Pat. No. 5,720,937, issued Feb. 24, 1998; U.S. Pat. No. 5,720,954, issued Feb. 24, 1998; U.S. Pat. No. 5,725,856, issued Mar. 10, 1998; U.S. Pat. No. 5,770,195, issued Jun. 23, 1998; U.S. Pat. No. 5,772,997, issued Jun. 30, 1998; PCT/US98/2626, published Dec. 10, 1998; and PCT/US99/06673, published Mar. 26, 1999, each of which patents and publications is herein incorporated by reference in its entirety.

II. Production of anti-ErbB2 Antibodies

A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention. The ErbB2 antigen to be used for production of antibodies may be, e.g., a soluble form of the extracellular domain of ErbB2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing ErbB2 at their cell surface (e.g. NIH-3T3 cells transformed to overexpress ErbB2; or a carcinoma cell line such as SKBR3 cells, see Stancovski et al., *PNAS* (*USA*) 88:8691-8695 [1991]) can be used to generate antibodies. Other forms of ErbB2 useful for generating antibodies will be apparent to those skilled in the art.

(i) Polyclonal Antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

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

(ii) Monoclonal Antibodies

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

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

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

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

anthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

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

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

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

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

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

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

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

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

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

(iii) Humanized and Human Antibodies

Methods for humanizing non-human antibodies are well known in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature,* 321:522-525 (1986); Riechmann et al., *Nature,* 332:323-327 (1988); Verhoeyen et al. *Science,* 239:1534-1536 [1988]), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some 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. Immnol.,* 151:2623 [1993]).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable

three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA,* 90:2551 (1993); Jakobovits et al., *Nature,* 362:255-258 (1993); Bruggermann et al. *Year in Immuno.,* 7:33 (1993). Human antibodies can also be derived from phage-display libraries (Hoogenboom et al., *J. Mol. Biol.,* 227:381 (1991); Marks et al., *J. Mol. Biol.,* 222:581-597 [1991]).

(iv) Antibody Fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan et al., *Science,* 229:81 [1985]). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al., *Bio/Technology* 10: 163-167 [1992]). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185.

(v) Bispecific Antibodies

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the ErbB2 protein. For example, one arm may bind an epitope in Domain 1 of ErbB2 such as the 7C2/7F3 epitope, the other may bind a different ErbB2 epitope, e.g. the 4D5 epitope. Other such antibodies may combine an ErbB2 binding site with binding site(s) for EGFR, ErbB3 and/or ErbB4. Alternatively, an anti-ErbB2 arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the ErbB2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express ErbB2. These antibodies possess an ErbB2-binding arm and an arm which binds the cytotoxic agent (e.g. saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

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

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

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

According to another approach described in WO96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. 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. Shalaby et al., *J. Exp. Med.,* 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

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

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

(vi) Screening for Antibodies with the Desired Properties

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

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

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

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

To 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 can be performed. According to this assay, SKBR3 cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillinstreptomycin. The SKBR3 cells are plated at 20,000 cells in a 35 mm cell culture dish (2 mls/35 mm dish). 2.5 μg/ml of the anti-ErbB2 antibody is added per dish. After six days, the number of cells, compared to untreated cells are counted using an electronic COULTER™ cell counter. Those antibodies which inhibit growth of the SKBR3 cells by 50-100% are selected for combination with the apoptotic antibodies as desired.

(vii) Effector Function Engineering

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

(viii) Immunoconjugates

The invention also pertains to immunoconjugates comprising the antibody described herein conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g. an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated anti-ErbB2 antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as his (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al. *Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 94/11026.

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

(ix) Immunoliposomes

The anti-ErbB2 antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., *Proc. Natl. Acad. Sci. USA,* 82:3688 (1985); Hwang et al., *Proc. Natl Acad. Sci. USA,* 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. 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). 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 covert it into its more active, cytotoxic form.

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

328: 457-458 [1987]). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

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

(xi) Antibody-Salvage Receptor Binding Epitope Fusions

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

A systematic method for preparing such an antibody variant having an increased in vivo half-life comprises several steps. The first involves identifying the sequence and conformation of a salvage receptor binding epitope of an Fc region of an IgG molecule. Once this epitope is identified, the sequence of the antibody of interest is modified to include the sequence and conformation of the identified binding epitope. After the sequence is mutated, the antibody variant is tested to see if it has a longer in vivo half-life than that of the original antibody. If the antibody variant does not have a longer in vivo half-life upon testing, its sequence is further altered to include the sequence and conformation of the identified binding epitope. The altered antibody is tested for longer in vivo half-life, and this process is continued until a molecule is obtained that exhibits a longer in vivo half-life.

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

The epitope preferably constitutes a region wherein any one or more amino acid residues from one or two loops of a Fc domain are transferred to an analogous position of the antibody fragment. Even more preferably, three or more residues from one or two loops of the Fc domain are transferred. Still more preferred, the epitope is taken from the CH2 domain of the Fc region (e.g., of an IgG) and transferred to the CH1, CH3, or V_H region, or more than one such region, of the antibody. Alternatively, the epitope is taken from the CH2 domain of the Fc region and transferred to the C_L region or V_L region, or both, of the antibody fragment.

In one most preferred embodiment, the salvage receptor binding epitope comprises the sequence (5' to 3'): PKNSSMISNTP (SEQ ID NO:3), and optionally further comprises a sequence selected from the group consisting of HQSLGTQ (SEQ ID NO:4), HQNLSDGK (SEQ ID NO:5), HQNISDGK (SEQ ID NO:6), or VISSHLGQ (SEQ ID NO:7), particularly where the antibody fragment is a Fab or $F(ab')_2$. In another most preferred embodiment, the salvage receptor binding epitope is a polypeptide containing the sequence(s) (5' to 3'): HQNLSDGK (SEQ ID NO:5), HQNISDGK (SEQ ID NO:6), or VISSHLGQ (SEQ ID NO:7) and the sequence: PKNSSMISNTP (SEQ ID NO:3).

(xii) 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 filter, for example, an 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 antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite 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]). 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 C_H3 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 SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following 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 quantitate the amount of specific rhuMAb HER2 (humanized anti-$p185^{HER2}$ 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-p185^{HER2} 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^{HER2} (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 are washed again with water as in previous steps.

Horse radish peroxidase-conjugate (HRP-conjugate, Goat anti-human IgG Fc 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 I 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_2O_2 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* 16 th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Preferred lyophilized anti-ErbB2 antibody formulations are described in WO 97/04801, expressly incorporated herein be 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-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16 th edition, Osol, A. Ed. (1980).

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

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(−)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C., resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S—S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

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 disor-

ders include benign or malignant tumors (e.g. renal, liver, kidney, bladder, breast, gastric, ovarian, colorectal, prostate, pancreatic, lung, vulval, thyroid, hepatic carcinomas; sarcomas; glioblastomas; and various head and neck tumors); leukemias and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The antibodies of the invention are administered to a human patient, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous 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 antracycline) is desired, the combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. 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 maybe 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 μg/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 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. 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 subcutaneous 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 instructions for use, including, e.g., a warning that the composition is not to be used in combination with anthacycline-type chemotherapeutic agent, e.g. doxorubicin or epirubicin.

Deposit of Materials

The following hybridoma cell lines have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md., USA (ATCC):

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

Further details of the invention are illustrated by the following non-limiting Examples.

EXAMPLES

Example 1

Preparation and Efficacy of HERCEPTIN® Anti-ErbB2 Antibody Materials and Methods

Anti-ErbB2 monoclonal antibody The anti-ErbB2 $IgG_1\kappa$ murine monoclonal antibody 4D5, specific for the extracellular domain of ErbB2, was produced as described in Fendly et al., *Cancer Research* 5o:1550-1558 (1990) and WO89/06692. Briefly, NIH 3T3/HER2-3_{400} cells (expressing approximately 1×10^5 ErbB2 molecules/cell) produced as described in Hudziak et al., *Proc. Natl. Acad. Sci.* (*USA*) 84:7159 (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 ml of the ErbB2 preparation and the splenocytes were fused with mouse myeloma line X63-Ag8.653. Hybridoma supernatants were screened for ErbB2-binding by ELISA and radioimmunoprecipitation. MOPC-21 (IgG1), (Cappell, Durham, N.C.), was used as an isotype-matched control.

The treatment was performed with a humanized version of the murine 4D5 antibody (HERCEPTIN® anti-ErbB2 antibody). The humanized antibody was engineered by inserting the complementarity determining regions of the murine 4D5 antibody into the framework of a consensus human immunoglobulin IgG_1 (IgG_1) (Carter et al., *Proc. Natl. Acad. Sci. USA* 89:4285-4289 [1992]). The resulting humanized anti-ErbB2 monoclonal antibody has high affinity for $p185^{HER2}$ (Dillohiation constant [K_d]=0.1 nmol/L), markedly inhibits, in vitro and in human xenografts, the growth of breast cancer cells that contain high levels of $p185^{HER2}$, induces antibody-dependent cellular cytotoxicity (ADCC), and has been found clinically active, as a single agent, in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior therapy. HERCEPTIN® anti-ErbB2 antibody is produced by a genetically engineered Chinese Hamster Ovary (CHO) cell line, grown in large scale, that secretes the antibody into the culture medium. The antibody is purified from the CHO culture media using standard chromatographic and filtration methods. Each lot of antibody used in this study was assayed to verify identity, purity, and potency, as well as to meet Food and Drug Administration requirements for sterility and safety.

Eligibility Criteria Patients had to fulfill all of the following criteria to be eligible for study admission:

- Metastatic breast cancer
- Overexpression of the ErbB2 (HER2) oncogene (2+ to 3+ as determined by immunohistochemistry or fluorescence in situ hybridization (FISH). [Tumor expression of ErbB2 can be determined by immunohistochemical analysis, as previously described (Slamon et al., [1987] and [1989], supra), of a set of thin sections prepared from the patient's paraffin-archived tumor blocks. The primary detecting antibody used is murine 4D5 MAb, which has the same CDRs as the humanized antibody used for the treatment. Tumors are considered to overexpress ErbB2 if at least 25% of tumor cells exhibit characteristic membrane staining for $p185^{HER2}$].
- 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:

- Prior cytotoxic chemotherapy for metastatic breast cancer
- Patients may have received prior hormonal therapy (e.g. tamoxifen) for metastatic disease or cytotoxic therapy in the adjuvant setting.
- Concomitant malignancy that has not been curatively treated
- A performance status of <60% on the Kamofsky scale

Pregnant or nursing women; women of childbearing potential, unless using effective contraception as determined by the investigator

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)

Use of investigational or unlicensed agents within 30 days prior to study entry

Clinically unstable or untreated metastases to the brain (e.g. requiring radiation therapy)

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

Anti-ErbB2 Antibody

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 (T, 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/m^2) 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/m^2) or epirubicin (75 mg/m^2) 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.

Paciltaxel (TAXOL®) was given at a dose of 175 mg/m^2 over 3 hours by intravenous administration. All patients receiving paclitaxel were premedicated with dexamethasone (or its equivalent) 20 mg×2, administered orally 12 and 6 hours prior to paclitaxel; diphenhydramine (or its equivalent) 50 mg, iv, administered 30 minutes prior to paclitaxel, and dimetidine (or another H_2 blocker) 300 mg, iv, administered 30 minutes prior to paclitaxel.

Response Criteria

Progressive Disease 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 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 1

HERCEPTIN ® Anti-ErbB2 Antibody Efficacy

	Enrolled	TTP(months)	RR(%)	AE(%)
CRx	234	5.5	36.2	66
CRx + H	235	8.6*	62.00**	69
AC	145	6.5	42.1	71
AC + H	146	9.0	64.9	68
T	89	4.2	25.0	59
T + H	89	7.1	57.3	70

*$p < 0.001$ by log-rank test;
**$p < 0.01$ by X^2 test;
CRx: chemotherapy;
AC: anthracycline/cyclophosphamide treatment;
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 ¾) 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. infu-

sions 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.

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

HERCEPTIN ® Anti-ErbB2 Antibody Trough and Peak Serum Concentrations for the First 8 Weeks of Treatment (μg/ml)

	Dose Number	n	Mean	SD	Minimun	Maximum
Peak	1	195	100.3	35.2	30.7	274.6
Trough		195	25.0	12.7	0.16	60.7
Peak	2	190	74.3	31.3	20.8	307.9
Trough		167	30.4	16.0	0.2	74.4
Peak	3	167	75.3	26.8	16.1	194.8
Trough		179	33.7	17.9	0.2	98.2
Peak	4	175	80.2	26.9	22.2	167
Trough		132	38.6	20.1	0.2	89.4
Peak	5	128	85.9	29.2	27.8	185.8
Trough		141	42.1	24.8	0.2	148.7
Peak	6	137	87.2	32.2	28.9	218.1
Trough		115	43.2	24.0	0.2	109.9
Peak	7	114	89.7	32.5	16.3	187.8
Trough		137	48.8	24.9	0.2	105.2
Peak	8	133	95.6	35.9	11.4	295.6

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 (n=40, 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=12.6). 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 of4 mg/kg weekly. The results of this preliminary human study indicated that an 8 mg/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 overexpresses the HER2 gene (BT-474M1, 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 3×10^6 BT474M1 cells suspended in Matrigel™. When tumor nodules reached a volume of approximately 100 mm^3, animals were randomized to 4 treatment groups. The groups were treated according to Table 3.

TABLE 3

Animal Groups and Doses for Comparison of I.V. Bolus and S.C. Infusion

Group, Dose, Antibody	Target Serum Conc. µg/ml	Route of Administration	Loading Dose (mg/kg)	Maintenance Dose
1 - Control, rhuMAb E25	20	IV LD and SC infusion	2.20	0.250 mg/ml (infusate)
2 - Low Dose SC rhuMAb HER2	1	IV LD and SC infusion	0.313	0.050 mg/ml (infusate)
3 - High Dose SC rhuMAb HER2	20	IV LD and SC infusion	6.25	1.00 mg/ml (infusate)
4 - IV Multi-Dose rhuMAb HER2	20 (trough)	IV LD and MD	4.00	2 mg/kg/week (IV bolus)

Serum Conc. = concentration in serum. LD = loading dose. MD = maintenance dose.
Infusate concentration was calculated to achieve targeted serum concentration using Alzet ® osmotic minipumps (Alza Corp., Palo Alto, CA).

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 nonrelevant antibody E25 (non-specific for HER2 receptor, but a member of the monoclonal IgG class) or test antibody HERCEPTIN® anti-ErbB2 anitbody 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 (just 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 6 to day 35 in the study for which data is tabulated below. Tumors were measured in three dimensions and volumes were expressed in mm^3. 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

Comparison of S.C. Infusion and I.V. Bolus Delivery

Treatment Group	Tumor Volume (mm^3), Day 35, (n = 14)	Tumor Volume (area under curve) Day 6-Day 35 (n = 13)	HERCEPTIN ® Serum Conc. (µg/ml), Day 27, (n = 3)
control s.c. infusion	764 ± 700	5650 ± 4700	4.16 ± 1.94
s.c. infusion (low dose)	80.6 ± 158	1610 ± 1250	2.11 ± 1.74
s.c. infusion (high dose)	31 ± 75.6	1440 ± 1140	22.1 ± 5.43
i.v. bolus dose*	49.7 ± 95.7	2150 ± 1480	21.7 ± 17.1**

s.c. = subcutaneous delivery; i.v. = intravenous delivery.
*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 innoculation as described in Example 3. Six days after tumor cell innoculation, the initial tumor measurement was performed. Seven days after tumor cell innoculation, 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

Animal Groups and Doses for Comparison of I.V. Bolus and S.C. Bolus Delivery

Group	Route of Administration	Loading Dose (mg/kg)	Maintenance Dose (mg/kg/week)	n
1 - Control rhuMAb E25	IV	8	4	10
2 - rhuMAb HER2	IV	2	1	10
3 - rhuMAb HER2	IV	4	2	10
4 - rhuMAb HER2	IV	8	4	10
5 - rhuMAb HER2	SC	4	2	10

IV = intraveneous; 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

IV versus SC Bolus Delivery: Serum HERCEPTIN ® Anti-ErbB2 Antibody Concentration

	Serum Concentration, μg/ml			
Treatment Group (delivery, MD)	Day 0 Mean (SD)	Day 7 Mean (SD)	Day 14 Mean (SD)	Day 21 Mean (SD)
1 - Control rhu MAb E25	0	25.9	34.6	38.5
(IV, 4 mg/kg)	(0)	(8.29)	(11.2)	(14.4)
2 - rhu MAb HER2	0	4.96	8.55	8.05
(IV, 1 mg/kg)	(0)	(3.79)	(5.83)	(4.67)
3 - rhu MAb HER2	0	13.4	18.9	22.6
(IV, 2 mg/kg)	(0)	(9.24)	(12.0)	(9.21)
4 - rhu MAb HER2	0	29.6	37.7	46.2
(IV, 4 mg/kg)	(0)	(13.5)	(14.4)	(13.8)
5 - rhu MAb HER2	0	12.5	16.9	17.6
(SC, 2 mg/kg)	(0)	(7.33)	(10.2)	(10.7)

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.

TABLE 7

Efficacy of HERCEPTIN ® Anti-ErbB2 Antibody Measured as a Change in Tumor Volume Comparing Intravenous Bolus and Subcutaneous Bolus Delivery, Mean (SD)

Treatment Group (Delivery, MD)	Tumor Vol. Day 6, mm^3	Tumor Vol. Day 28, mm^3	Tumor Vol. Day 31, mm^3	Day 6-Day 31* Area Under Curve Tumor Vol., mm^3	Tumor Growth Rate on Log (TM + 1)
1-IV Control	321	1530	1630	13600	0.0660
	(190)	(1040)	(1170)	(7230)	(0.0200)
2-IV Herceptin	297	175	151	4690	−0.0505
1 mg/kg	(130)	(215)	(188)	(1400)	(0.142)
3-IV Herceptin	269	75.7	73.6	3510	−0.0608
2 mg/kg	(129)	(92.4)	(84.5)	(1220)	(0.110)
4-IV Herceptin	272	25.3	25.8	2880	−0.0810
4 mg/kg	(117)	(75.9)	(72.9)	(1230)	(0.0859)
5-SC Herceptin	268	76.2	90.4	3230	−0.0304
2 mg/kg	(117)	(98.8)	(105)	(1440)	(0.104)

N = 10 for each data point. TM = tumor measurement. IV = intravenous. SC = subcutaneous. MD = maintenance dose. Tumor Vol. = tumor volume, mm^3.
*Day 17 excluded due to measurement error.
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.

FIGS. **4**A and **4**B are graphical plots of changes in tumor volume over time, some of which data is found in Table 7. FIG. **4**A is a linear plot of tumor volume versus time. FIG. **4**B is a semilogarithmic plot of the same data, allowing the test points be viewed more clearly. The data in Table 7 and FIGS. **4**A and **4**B indicate that, although a dose-related response was not observed between HERCEPTIN-treated groups, dosing by subcutaneous bolus was as effective as intravenous bolus dosing and achieved similar trough serum concentrations.

Example 5

Regimens for Intravenous and Subcutaneous Delivery of Anti-ErbB2 Antibody

According to the invention, methods of anti-ErbB2 antibody (e.g., HERCEPTIN®) delivery comprise greater front loading of the drug to achieve a 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 of equal or smaller amount. An advantage of the methods of the invention is that the maintenance dosing may be less frequent and/or delivered by subcutaneous injection, making the treatment regimens of the invention convenient and cost-effective for the patient and medical professionals administering the antibody. In addition, a subcutaneous maintenance dose regimen may be interrupted by intravenous dosing (such as infusion) when the patient's chemotherapy requires delivery of other drugs by intravenous injection.

To test the following dosage regimens, human subjects are selected according to the criteria disclosed in Example 1, above. The number of initial doses is one or more doses 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 1 day or less. The number of maintenance doses may be one or more doses sufficient to achieve suppression of disease symptoms, such as a decrease in tumor volume. The maintenance doses are 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 delivery subsequent maintenance doses.

In another method, an initial (front loading) dose of 8 mg/kg HERCEPTIN® anti-ErbB2 antibody is delivered by intravenous injection (infusion 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 injection (infusion 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 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 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 example, 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 is optionally and preferably deliverable 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 anthrocycline 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 175 mg/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/m^2/week. According to another embodiment of the invention, the taxoid is docetaxel and is administered at a dose of 30-70 mg/m^2/week.

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/m^2 every three weeks). Simulation of the proposed treatment regimen suggests that the trough serum concentrations will be 17 mcg/ml, in the range (10-20 mcg/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) Females24 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 24 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 spermicidal 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 3.0×10^9/l
   Granulocytes less than 1.5×10^9/l
   Platelets less than 100×10^9/l
8) Any of the following abnormal baseline liver function tests:
   Serum bilirubin greater than 1.5×ULN (upper normal limit)
   ALT and/or AST greater than 2.5×ULN (greater than 4.0×ULN if liver or bone metastasis)
   Alkaline phosphatase greater than 2.5×ULN (greater than 4.0×ULN if liver or bone metastasis)
9) The following abnormal baseline renal function tests:
   serum creatinine greater than 1.5×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/m^2 IV every 3 weeks×6 cycles as a 3-hour infusion.

NOTE: On the first cycle of treatment, paclitaxel will be dosed 8 hours prior to HERCEPTIN® to determine the PK of paclitaxel alone. HERCEPTIN® will be administered 8 hours post-paclitaxel for the 1st cycle only. In subsequent treatment cycles, HERCEPTIN® will be administered prior to paclitaxel.

The total duration of this study is 18 weeks. Study subjects will receive up to 6 total HERCEPTIN® doses. After the last subject has received the last cycle of paclitaxel, data collection for safety and pharmacokinetic analysis will stop, and the study will close to protocol specified treatment. Study subjects may continue to receive the HERCEPTIN® +/− paclitaxel at the discretion of the investigator.

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.

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Ile Ala His Asn Gln Val Arg Gln Val Pro Leu Gln Arg Leu Arg
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Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro Val Thr
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Asn Ala Arg His Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln
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Lys Asp Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys
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35 40 45

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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20 25 30

Asp Tyr Thr Met Asp Trp Val Lys Gln Ser His Gly Lys Ser Leu
35 40 45

Glu Trp Ile Gly Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
50 55 60

Asn Gln Arg Phe Lys Gly Lys Ala Ser Leu Thr Val Asp Arg Ser
65 70 75

Ser Arg Ile Val Tyr Met Glu Leu Arg Ser Leu Thr Phe Glu Asp
80 85 90

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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

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

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
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Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
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Ile Lys


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

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

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Ile Lys
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Glu Trp Val Ala Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
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Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
65 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 Gly Arg Val Gly Tyr Ser Leu
95 100 105

Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

The invention 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 two weeks; and

further comprising administering an effective amount of a chemotherapeutic agent to the patient.

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. 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.

17. The method of claim **16**, wherein said cancer is breast cancer.

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

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

20. The method of claim **19**, wherein said antibody binds to epitope 4D5 within the ErbB2 extracellular domain sequence.

21. The method of claim **20**, wherein said antibody is a humanized 4D5 anti-ErbB2 antibody.

22. The method of claim **1**, wherein the chemotherapeutic agent is a taxoid.

23. The method of claim **22**, wherein said taxoid is paclitaxel or docetaxel.

24. The method of claim **1**, wherein the effective amount of the anti-ErbB2 antibody and the effective amount of the chemotherapeutic agent as a combination is lower than the sum of the effective amounts of said anti-ErbB2 antibody and said chemotherapeutic agent, when administered individually, as single agents.

25. The method of claim **1**, wherein the chemotherapeutic agent is an anthracycline.

26. The method of claim **25**, wherein the anthracycline is doxorubicin or epirubicin.

27. The method of claim **25**, wherein the method further comprises administration of a cardioprotectant.

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

29. 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 one 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, and further comprising administering an effective amount of a chemotherapeutic agent to the patient.

30. 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 from each other in time by at least about two weeks, and further comprising administering an effective amount of a chemotherapeutic agent to the patient.

31. The method of claim **30**, wherein the first dose and a first subsequent dose are separated from each other in time by at least about three weeks.

32. The method of claim **30**, wherein the first dose and subsequent doses are each from about 2 mg/kg to about 16 mg/kg.

33. The method of claim **32**, wherein the first dose and subsequent doses are each from about 4 mg/kg to about 12 mg/kg.

34. The method of claim **33**, wherein the first dose and subsequent doses are each from about 6 mg/kg to about 12 mg/kg.

35. The method of claim **30**, wherein from about two to about ten subsequent doses of the antibody are administered to the patient.

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

37. The method of claim **30**, wherein the two or more subsequent doses are each from about 2 mg/kg to about 16 mg/kg.

38. The method of claim **30**, wherein the two or more subsequent doses are each from about 4 mg/kg to about 12 mg/kg.

39. The method of claim **30**, wherein the two or more subsequent doses are each from about 6 mg/kg to about 12 mg/kg.

40. The method of claim **30**, wherein the chemotherapeutic agent is a taxoid.

* * * * *

US010160811B2

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

(10) **Patent No.: US 10,160,811 B2**
(45) **Date of Patent: *Dec. 25, 2018**

(54) **TREATMENT WITH ANTI-ERBB2 ANTIBODIES**

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(72) Inventors: **Sharon A. Baughman**, Ventura, CA (US); **Steven Shak**, Burlingame, CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 843 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **14/073,659**

(22) Filed: **Nov. 6, 2013**

(65) **Prior Publication Data**

US 2014/0079692 A1 Mar. 20, 2014

Related U.S. Application Data

(60) Continuation of application No. 13/415,271, filed on Mar. 8, 2012, now abandoned, which is a continuation of application No. 13/167,599, filed on Jun. 23, 2011, now abandoned, which is a continuation of application No. 11/443,943, filed on May 31, 2006, now abandoned, which is a division of application No. 10/600,152, filed on Jun. 20, 2003, now Pat. No. 7,371,379, which is a division of application No. 09/648,067, filed on Aug. 25, 2000, now Pat. No. 6,627,196.

(60) Provisional application No. 60/213,822, filed on Jun. 23, 2000, provisional application No. 60/151,018, filed on Aug. 27, 1999.

(51) **Int. Cl.**

C07K 16/30	(2006.01)
A61K 39/395	(2006.01)
C07K 16/32	(2006.01)
A61K 45/06	(2006.01)
A61K 31/337	(2006.01)
A61K 39/00	(2006.01)
A61K 38/00	(2006.01)

(52) **U.S. Cl.**

CPC ***C07K 16/30*** (2013.01); ***A61K 31/337*** (2013.01); ***A61K 39/395*** (2013.01); ***A61K 39/39558*** (2013.01); ***A61K 45/06*** (2013.01); ***C07K 16/32*** (2013.01); *A61K 38/00* (2013.01); *A61K 2039/505* (2013.01); *A61K 2039/54* (2013.01); *A61K 2039/545* (2013.01)

(58) **Field of Classification Search**

CPC A61K 47/48384

See application file for complete search history.

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(57) **ABSTRACT**

The present invention concerns dosages for treatment of human cancer patients with an anti-Epidermal Growth Factor Receptor (EGFR) antibody.

12 Claims, 5 Drawing Sheets

Specification includes a Sequence Listing.

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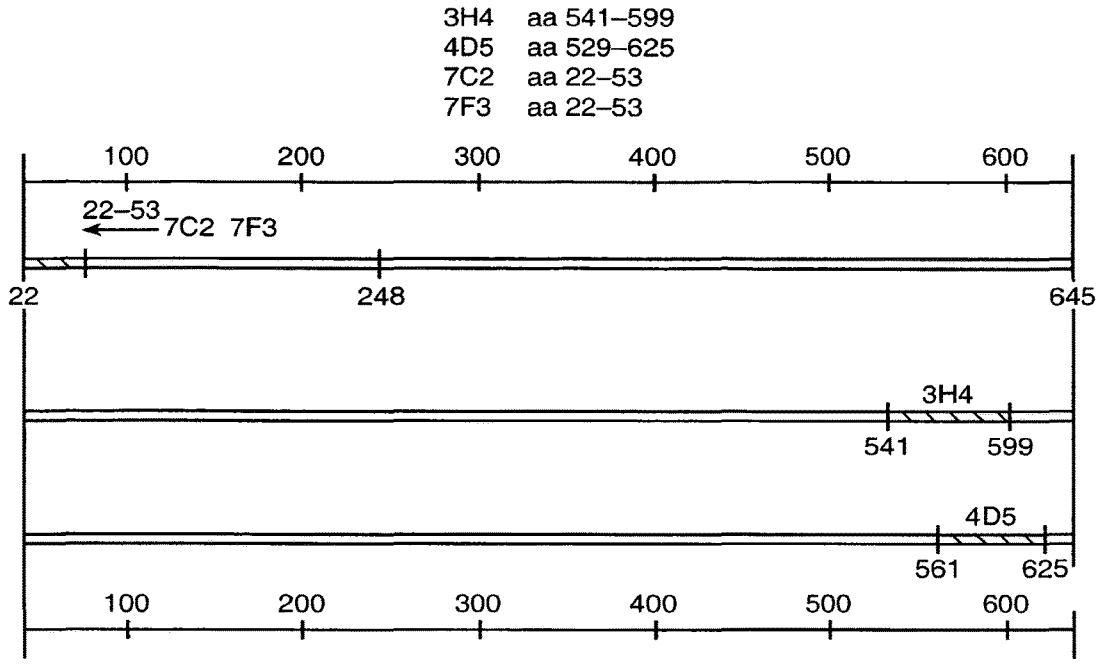

3H4 epitope (SEQ ID NO:8) 58 residues

VEECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVAR

541 **599**

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

LPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQP

561 **625**

FIG._1

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

FIG._2

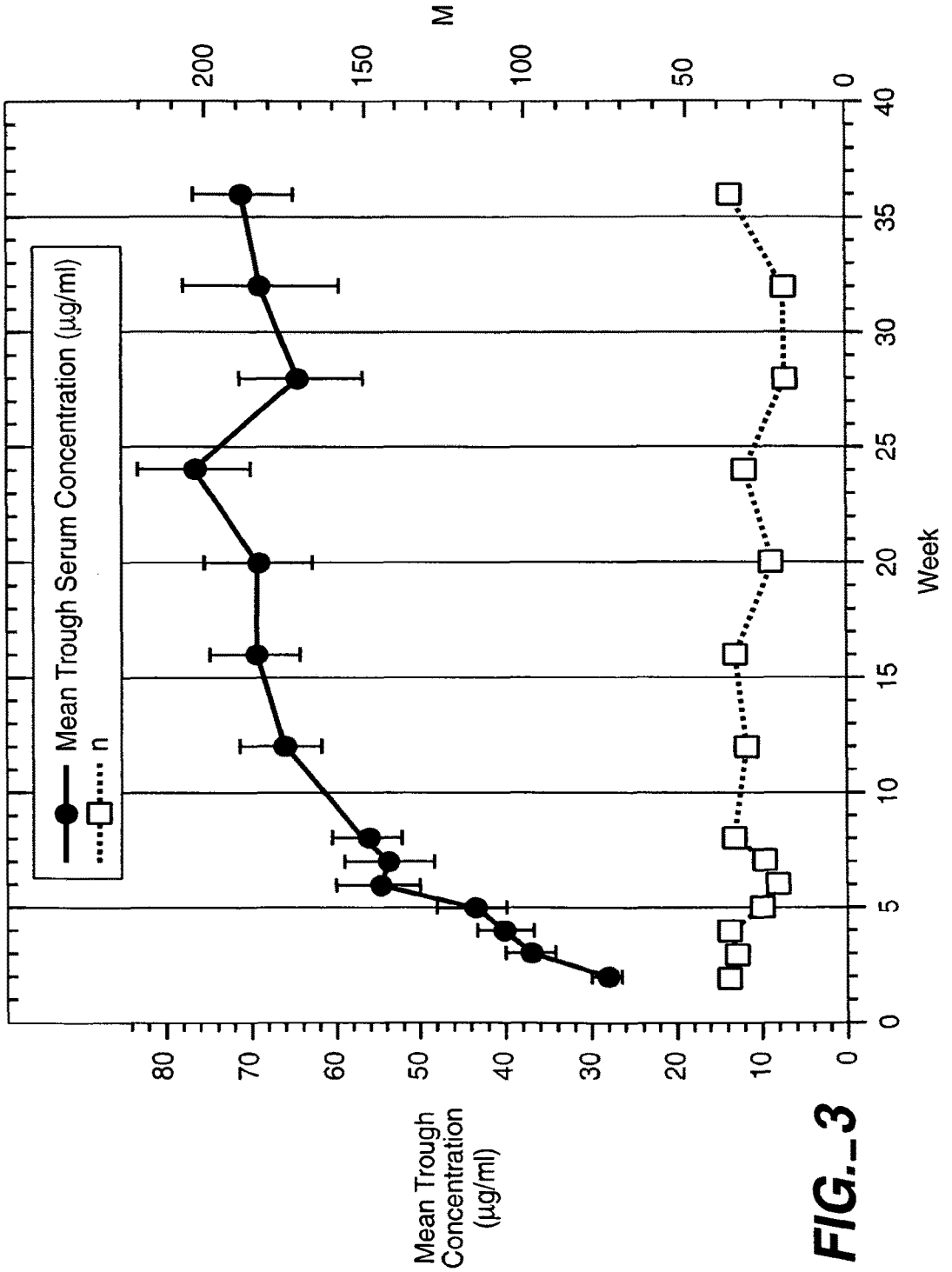
Mean Trough Serum Concentration (µg/ml)
n
Mean Trough Concentration (µg/ml)
Week
M
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25
30
35
40
0
50
100
150
200

FIG._3

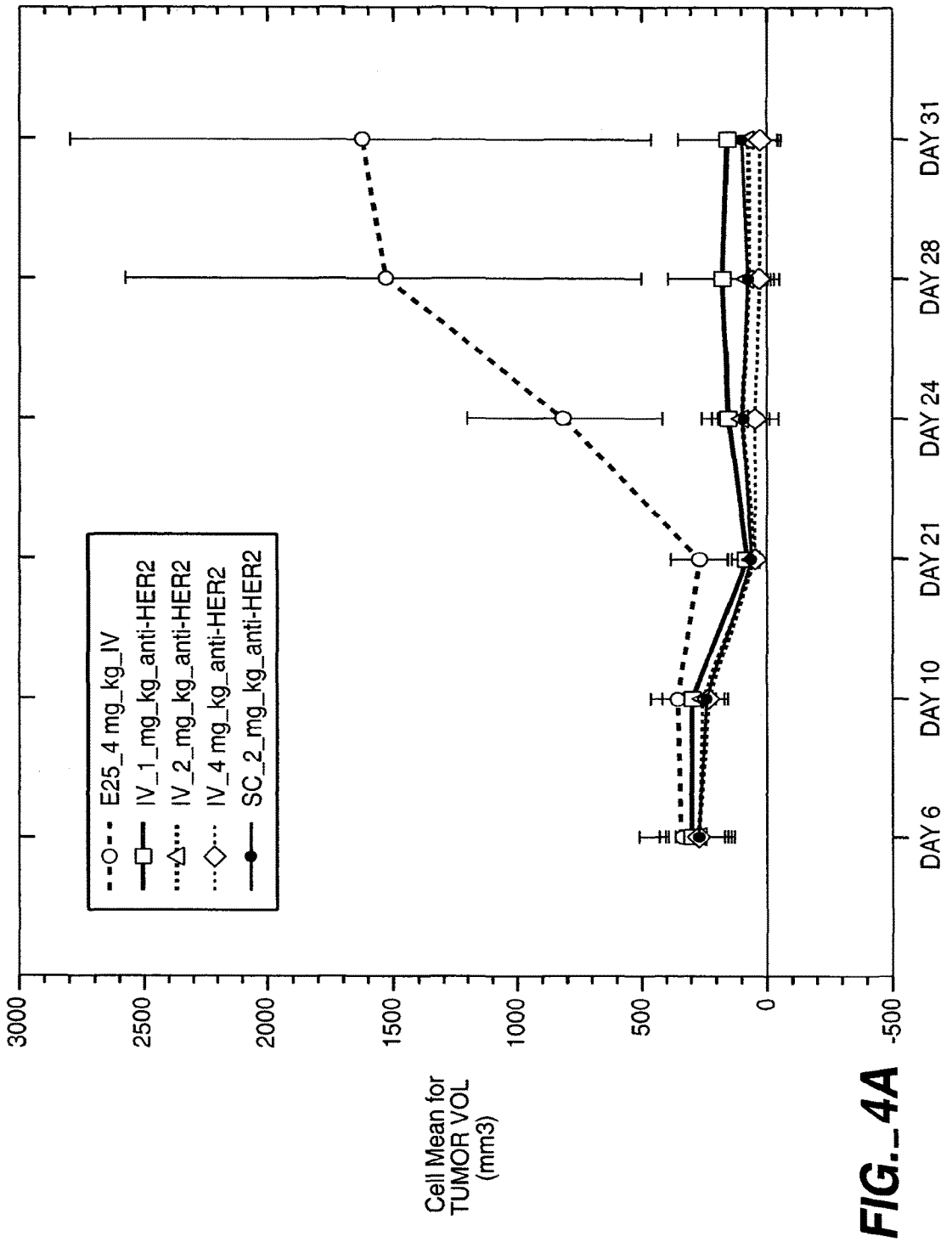
E25_4 mg_kg_IV
IV_1_mg_kg_anti-HER2
IV_2_mg_kg_anti-HER2
IV_4 mg_kg_anti-HER2
SC_2_mg_kg_anti-HER2
3000
2500
2000
1500
1000
500
0
-500
Cell Mean for TUMOR VOL (mm3)
DAY 6
DAY 10
DAY 21
DAY 24
DAY 28
DAY 31

FIG._4A

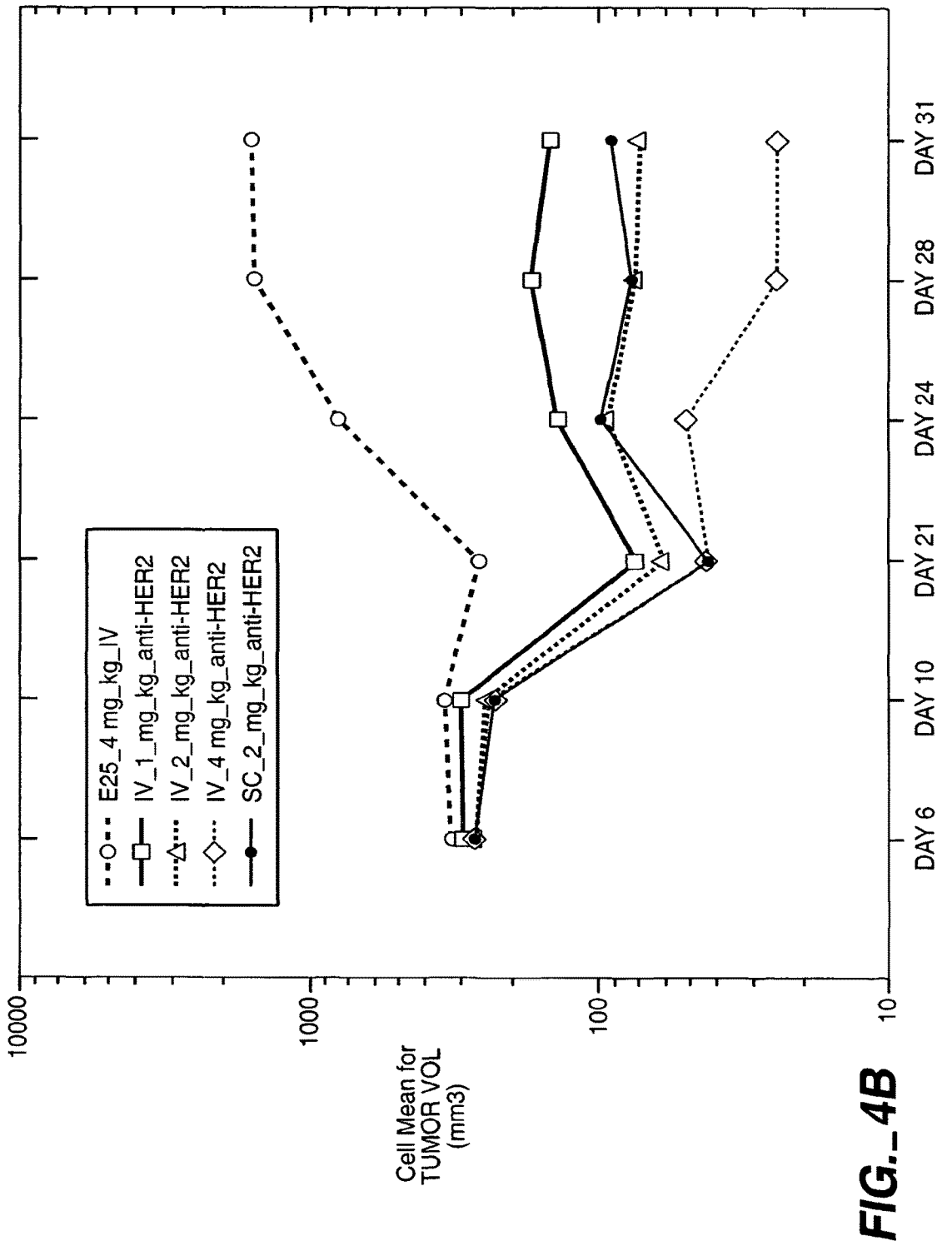
E25_4 mg_kg_IV
IV_1_mg_kg_anti-HER2
IV_2_mg_kg_anti-HER2
IV_4 mg_kg_anti-HER2
SC_2_mg_kg_anti-HER2
Cell Mean for TUMOR VOL (mm3)
10000
1000
100
10
DAY 6
DAY 10
DAY 21
DAY 24
DAY 28
DAY 31

FIG._4B

VARIABLE LIGHT

```
          1       10        20          30              40
2C4       DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIGVA----] WYQQRP
           **    **** *      *                        *
574       DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA----] WYQQKP
                                   *   *  ****
hum kI    DIQMTQSPSSLSASVGDRVTITC [RASQSVSTSSYSYMH] WYQQKP

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

                   90          100
2C4       EDLAVYYC [QQYYIYPYT] FGGGTKLEIK (SEQ ID NO:10)
            * *                  *   *
574       EDFATYYC [QQYYIYPYT] FGQGTKVEIK (SEQ ID NO:12)
                       ***
hum kI    EDFATYYC [QQYNSLPYT] FGQGTKVEIK (SEQ ID NO:14)
```

FIG._5A

VARIABLE HEAVY

```
          1       10        20          30          40
2C4       EVQLQQSGPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS
              **  **  *  * ***  **                   *
574       EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA
                                       ** * *
humIII    EVQLVESGGGSVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                    50        60          70        80
2C4       HGKSLEWIG [DVNPNSGGSIYNQRFKG] KASLTVDRSSRIVYM
          *  *   **                     *** *    **** *
574       PGKGLEWVA [DVNPNSGGSIYNQRFKG] RFTLSVDRSKNTLYL
                    *  ******  ** ****      * * *
humIII    PGKGLEWVS [VISGDGGSTYYADSVKG] RFTISRDDSKNTLYL

                     90         100          110
2C4       ELRSLTFEDTAVYYCAR [NLGPSFYFDY] WGQGTTLVTSS (SEQ ID NO:11)
          ***  **                              *
574       QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS (SEQ ID NO:13)
                            ** ***
humIII    QMNSLRAEDTAVYYCAR [GRGGGS--DY] WGQGTLVTVSS (SEQ ID NO:15)
```

FIG._5B

TREATMENT WITH ANTI-ERBB2 ANTIBODIES

RELATED APPLICATIONS

This application is divisional of U.S. Ser. No. 10/600,152 filed Jun. 20, 2003, which is a divisional of U.S. Ser. No. 09/648,067 filed Aug. 25, 2000 (now U.S. Pat. No. 6,627,196), which claims priority under 35 USC 119(e) to provisional application Nos. 60/151,018, filed Aug. 27, 1999 and 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 ($p185^{HER2}$) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer (Slamon et al., *Science* 235:177-182 [1987]; Slamon et al., *Science* 244:707-712 [1989]).

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

Antibodies directed against human erbB2 protein products and proteins encoded by the rat equivalent of the erbB2 gene (neu) have been described. Drebin et al., *Cell* 41:695-706 (1985) refer to an IgG2a monoclonal antibody which is directed against the rat neu gene product. This antibody called 7.16.4 causes down-modulation of cell surface p185 expression on B104-1-1 cells (NIH-3T3 cells transfected with the neu proto-oncogene) and inhibits colony formation of these cells. In Drebin et al. *PNAS* (*USA*) 83:9129-9133 (1986), the 7.16.4 antibody was shown to inhibit the tumorigenic growth of neu-transformed NIH-3T3 cells as well as rat neuroblastoma cells (from which the neu oncogene was initially isolated) implanted into nude mice. Drebin et al. in *Oncogene* 2:387-394 (1988) discuss the production of a panel of antibodies against the rat neu gene product. All of the antibodies were found to exert a cytostatic effect on the growth of neu-transformed cells suspended in soft agar. Antibodies of the IgM, IgG2a and IgG2b isotypes were able to mediate significant in vitro lysis of neu-transformed cells in the presence of complement, whereas none of the antibodies were able to mediate high levels of antibody-dependent cellular cytotoxicity (ADCC) of the neu-transformed cells. Drebin et al. *Oncogene* 2:273-277 (1988) report that mixtures of antibodies reactive with two distinct regions on the p185 molecule result in synergistic anti-tumor effects on neu-transformed NIH-3T3 cells implanted into nude mice. Biological effects of anti-neu antibodies are reviewed in Myers et al., *Meth. Enzym.* 198:277-290 (1991). See also WO94/22478 published Oct. 13, 1994. Hudziak et al., *Mol. Cell. Biol.* 9(3): 1165-1172 (1989) describe the generation of a panel of anti-ErbB2 antibodies which were characterized using the human breast tumor cell line SKBR3. Relative cell proliferation of the SKBR3 cells following exposure to the antibodies was determined by crystal violet staining of the monolayers after 72 hours. Using this assay, maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel, including 7C2 and 7F3, reduced cellular proliferation to a lesser extent in this assay. Hudziak et al. conclude that the effect of the 4D5 antibody on SKBR3 cells was cytostatic rather than cytotoxic, since SKBR3 cells resumed growth at a nearly normal rate following removal of the antibody from the medium. The antibody 4D5 was further found to sensitize $p185^{erbB2}$-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also WO89/06692 published 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); Vitetta et al. *Cancer Research* 54:5301-5309 (1994); Sliwkowski et al. *J. Biol. Chem.* 269(20):14661-14665 (1994); Scott et al. *J. Biol. Chem.* 266:14300-5 (1991); and D'souza et al. *Proc. Natl. Acad. Sci.* 91:7202-7206 (1994).

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

McKenzie et al. *Oncogene* 4:543-548 (1989) generated a panel of anti-ErbB2 antibodies with varying epitope specificities, including the antibody designated TA1. This TA1 antibody was found to induce accelerated endocytosis of ErbB2 (see Maier et al. *Cancer Res.* 51:5361-5369 [1991]). Bacus et al. *Molecular Carcinogenesis* 3:350-362 (1990) reported that the TA1 antibody induced maturation of the breast cancer cell lines AU-565 (which overexpresses the 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 fibroblasts 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 fibroblasts overexpressing human ErbB2 was assessed. As observed in their earlier work, N28 accelerated tumor growth, whereas N12 and N29 significantly inhibited growth of the ErbB2-expressing cells. Partial tumor inhibition was also observed with the N24 antibody. Bacus et al. also tested the ability of the antibodies to promote a mature phenotype in the human breast cancer cell lines AU-565 and MDA-MB453 (which overexpress ErbB2) as well as MCF-7 (containing low levels of the receptor). Bacus et al. saw a correlation between tumor inhibition in vivo and cellular differentiation; the tumor-stimulatory antibody N28 had no effect on differentiation, and the tumor inhibitory action of the N12, N29 and N24 antibodies correlated with the extent of differentiation they induced.

Xu et al. *Int. J. Cancer* 53:401-408 (1993) evaluated a panel of anti-ErbB2 antibodies for their epitope binding specificities, as well as their ability to inhibit anchorage-independent and anchorage-dependent growth of SKBR3 cells (by individual antibodies and in combinations), modulate cell-surface ErbB2, and inhibit ligand stimulated anchorage-independent growth. See also WO94/00136 published Jan. 6, 1994 and Kasprzyk et al. *Cancer Research* 52:2771-2776 (1992) concerning anti-ErbB2 antibody combinations. Other anti-ErbB2 antibodies are discussed in Hancock et al. *Cancer Res.* 51:4575-4580 (1991); Shawver et al. *Cancer Res.* 54:1367-1373 (1994); Arteaga et al. *Cancer Res.* 54:3758-3765 (1994); and Harwerth et al. *J. Biol. Chem.* 267:15160-15167 (1992).

A recombinant humanized anti-ErbB2 monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2, 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 (Baselga 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 Chamness, *Gene* 159:19-27 [1995]; and Hynes and Stern, *Biochim Biophys Acta* 1198:165-184 [1994]), and has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluoruracil) and anthracyclines (Baselga et al., *Oncology* 11(3 Suppl 1):43-48 [1997]). However, despite the association of ErbB2 overexpression with poor prognosis, the odds of HER2-positive patients responding clinically to treatment with taxanes were greater than three times those of HER2-negative patients (Ibid). rhuMab HER2 was shown to enhance the activity of paclitaxel (TAXOL®) and doxorubicin against breast cancer xenografts in nude mice injected with BT-474 human breast adenocarcinoma cells, which express high levels of HER2 (Baselga et al., *Breast Cancer, Proceedings of ASCO*, Vol. 13, Abstract 53 [1994]).

SUMMARY OF THE INVENTION

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 μg/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 8 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 about 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. Generally, two or more subsequent doses (e.g. from about two to about ten subsequent doses) of the antibody are administered to the patient, and those subsequent doses are preferably 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). The two or more subsequent doses may each be from about 2 mg/kg to about 16 mg/kg; or from about 4 mg/kg to about 12 mg/kg; or from about 6 mg/kg to about 12 mg/kg. 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.

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. The invention additionally provides an article of manufacture, comprising a container, a composition within the container comprising an anti-ErbB 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 label on or associated with the container that indicates that the composition can be used for treating a condition characterized by overexpression of ErbB2 receptor, and a package insert containing instructions to avoid the use of anthracycline-type chemotherapeutics in combination with the composition. According to the invention, the 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 including, but not limited to, paclitaxel and docetaxel. In another embodiment, the chemotherapeutic agent is an anthracyline derivative including, but not limited to, doxorubicin or epirubicin. In still another embodiment of the invention, treatment with an anti-ErbB2 antibody and an anthracycline derivative further includes administration of 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 composition and a chemotherapeutic agent to a patient. In another embodiment, the chemotherapeutic agent is other than an anthracycline, and is preferably a taxoid, such as TAXOL®. In still another embodiment, the chemotherapeutic agent is an anthracycline, including but not limited to, doxorubicin or epirubicin. In yet another embodiment, the chemotherapeutic agent is an anthracycline and the package insert further instructs the user to administer a cardioprotectant.

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 huMAb4D5-8 (HERCEPTIN® anti-ErbB2 antibody); or monoclonal antibody 2C4, e.g., humanized 2C4. The antibody may be an intact antibody (e.g., an intact IgG_1 antibody) or an antibody fragment (e.g., a Fab, $F(ab')_2$, diabody, and the like). The variable light chain and variable heavy chain regions of humanized anti-ErbB2 antibody 2C4 are shown in FIGS. **5**A and **5**B.

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.* 125(6):1395-1406 [June 1994]). The anti-proliferative MAbs 4D5 and 3H4 bind adjacent to the transmembrane domain. The various ErbB2-ECD truncations or point mutations were prepared from cDNA using polymerase chain reaction technology. The ErbB2 mutants were expressed as gD fusion proteins in a mammalian expression plasmid. This expression plasmid uses the cytomegalovirus promoter/enhancer with SV40 termination and polyadenylation signals located downstream of the inserted cDNA. Plasmid DNA was transfected into 293S cells. One day following transfection, the cells were metabolically labeled overnight in methionine and cysteine-free, low glucose DMEM containing 1% dialyzed fetal bovine serum and 25 μCi each of ^{35}S methionine and ^{35}S cysteine. Supernatants were harvested either the ErbB2 MAbs or control antibodies were added to the supernatant and incubated 2-4 hours at 4° C. The complexes were precipitated, applied to a 10-20% Tricine SDS gradient gel and electrophoresed at 100 V. The gel was electroblotted onto a membrane and analyzed by autoradiography. SEQ ID NOs:8 and 9 depict the 3H4 and 4D5 epitopes, respectively.

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

FIG. **3** is a graph of anti-ErbB2 antibody (HERCEPTIN®) trough serum concentration (μg/ml, mean±SE, dark circles) by week from week 2 through week 36 for ErbB2 overexpressing patients treated with HERCEPTIN® anti-ErbB2 antibody at 4 mg/kg initial dose, followed by 2 mg/kg weekly. The number of patients at each time point is represented by "n" (white squares).

FIG. **4**A is a linear plot of tumor volume changes over time in mice treated with HERCEPTIN® anti-ErbB2 antibody. FIG. **4**B is a semi-logarithmic plot of the same data as in FIG. **4**A such that the variation in tumor volume for the treated animals is observed more readily.

FIGS. **5**A and **5**B depict alignments of the amino acid sequences of the variable light (V_L) (FIG. **5**A) and variable heavy (V_H) (FIG. **5**B) domains of murine monoclonal antibody 2C4 (SEQ ID Nos. 10 and 11, respectively); V_L and V_H domains of humanized Fab version 574 (SEQ ID Nos. 12 and 13, respectively), and human V_L and V_H consensus frameworks (hum κ1, light kappa subgroup I; humIII, heavy subgroup III) (SEQ ID Nos. 14 and 15, respectively). Asterisks identify differences between humanized Fab version 574 and murine monoclonal antibody 2C4 or between humanized Fab version 574 and the human framework. Complementarity Determining Regions (CDRs) are in brackets. Humanized Fab version 574, with the changes ArgH71Val, AspH73Arg and IleH69Leu, appears to have binding restored to that of the original chimeric 2C4 Fab fragment. Additional FR and/or CDR residues, such as L2, L54, L55, L56, H35 and/or H48, may be modified (e.g.

substituted as follows—IleL2Thr; ArgL54Leu; TyrL55Glu; ThrL56Ser; AspH35Ser; and ValH48Ile) in order to further refine or enhance binding of the humanized antibody. Alternatively, or additionally, the humanized antibody may be affinity matured in order to further improve or refine its affinity and/or other biological activities.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

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. *Ann. Rev. Biochem.* 56:881-914 (1987), including variants thereof (e.g. a deletion mutant EGFR as in Humphrey et al. *PNAS* (*USA*) 87:4207-4211 (1990)). erbB1 refers to the gene encoding the EGFR protein product. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, U.S. Pat. No. 4,943,533, Mendelsohn et al.) and variants thereof, such as chimerized 225 (C225) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.).

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

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

The terms "HER2", "ErbB2" "c-Erb-B2" are used interchangeably. Unless indicated otherwise, the terms "ErbB2" "c-Erb-B2" and "HER2" when used herein refer to the human protein, and "erbB2," "c-erb-B2," and "her2" refer to human gene. The human erbB2 gene and ErbB2 protein are, for example, described in Semba et al., *PNAS* (*USA*) 82:6497-6501 (1985) and Yamamoto et al. *Nature* 319:230-234 (1986) (Genebank accession number X03363). ErbB2 comprises four domains (Domains 1-4).

The "epitope 4D5" is the region in the extracellular domain of ErbB2 to which the antibody 4D5 (ATCC CRL 10463) binds. This epitope is close to the transmembrane region of ErbB2. To screen for antibodies which bind to the 4D5 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed (see FIG. **1**) to assess whether the antibody binds to the 4D5 epitope of ErbB2 (i.e. any one or more residues in the region from about residue 529, e.g. about residue 561 to about residue 625, inclusive).

The "epitope 3H4" is the region in the extracellular domain of ErbB2 to which the antibody 3H4 binds. This epitope is shown in FIG. **1**, and includes residues from about 541 to about 599, inclusive, in the amino acid sequence of ErbB2 extracellular domain.

The "epitope 7C2/7F3" is the region at the N-terminus of the extracellular domain of ErbB2 to which the 7C2 and/or 7F3 antibodies (each deposited with the ATCC, see below) bind. To screen for antibodies which bind to the 7C2/7F3 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to establish whether the antibody binds to the 7C2/7F3 epitope on ErbB2 (i.e. any one or more of residues in the region from about residue 22 to about residue 53 of ErbB2; SEQ ID NO:2).

The term "induces cell death" or "capable of inducing cell death" refers to the ability of the antibody to make a viable cell become nonviable. The "cell" here is one which expresses the ErbB2 receptor, especially where the cell overexpresses the ErbB2 receptor. A cell which "overexpresses" ErbB2 has significantly higher than normal ErbB2 levels compared to a noncancerous cell of the same tissue type. Preferably, the cell is a cancer cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SKBR3, BT474, Calu 3, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Cell death in vitro may be determined in the absence of complement and immune effector cells to distinguish cell death induced by antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Thus, the assay for cell death may be performed using heat inactivated serum (i.e. in the absence of complement) and in the absence of immune effector cells. To determine whether the antibody is able to induce cell death, loss of membrane integrity as evaluated by uptake of propidium iodide (PI), trypan blue (see Moore et al. Cytotechnology 17:1-11 [1995]) or 7AAD can be assessed relative to untreated cells. Preferred cell death-inducing antibodies are those which induce PI uptake in the "PI uptake assay in BT474 cells".

The phrase "induces apoptosis" or "capable of inducing apoptosis" refers to the ability of the antibody to induce programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is one which overexpresses the ErbB2 receptor. Preferably the "cell" is a tumor cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SKBR3, BT474, Calu 3 cell, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering as disclosed in the example herein; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is

one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an "annexin binding assay using BT474 cells" (see below).

Sometimes the pro-apoptotic antibody will be one which blocks HRG binding/activation of the ErbB2/ErbB3 complex (e.g. 7F3 antibody). In other situations, the antibody is one which does not significantly block activation of the ErbB2/ErbB3 receptor complex by HRG (e.g. 7C2). Further, the antibody may be one like 7C2 which, while inducing apoptosis, does not induce a large reduction in the percent of cells in S phase (e.g. one which only induces about 0-10% reduction in the percent of these cells relative to control).

The antibody of interest may be one like 7C2 which binds specifically to human ErbB2 and does not significantly cross-react with other proteins such as those encoded by the 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).

"Heregulin" (HRG) when used herein refers to a polypeptide which activates the ErbB2-ErbB3 and ErbB2-ErbB4 protein complexes (i.e. induces phosphorylation of tyrosine residues in the complex upon binding thereto). Various heregulin polypeptides encompassed by this term are disclosed in Holmes et al., *Science,* 256:1205-1210 (1992); WO 92/20798; Wen et al., *Mol. Cell. Biol.,* 14(3):1909-1919 (1994); and Marchionni et al., *Nature,* 362:312-318 (1993), for example. The term includes biologically active fragments and/or variants of a naturally occurring HRG polypeptide, such as an EGF-like domain fragment thereof (e.g. $HRG\beta1_{177-244}$).

The "ErbB2-ErbB3 protein complex" and "ErbB2-ErbB4 protein complex" are noncovalently associated oligomers of the ErbB2 receptor and the ErbB3 receptor or ErbB4 receptor, respectively. The complexes form when a cell expressing both of these receptors is exposed to HRG and can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski et al., *J. Biol. Chem.,* 269(20):14661-14665 (1994).

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

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

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the 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 are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity.

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

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

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

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

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different

classes of immunoglobulins are called α, δ, ∈, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

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

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

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., *Nature,* 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. 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 a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; 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 optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature,* 321:522-525 (1986); Reichmann et al., *Nature,* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.,* 2:593-596 (1992). The humanized antibody includes a PRIMATIZED™ antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

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

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

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

As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of

treatment include those already with the disorder as well as those in which the disorder is to be prevented.

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

A "disorder" is any condition that would benefit from treatment with the anti-ErbB2 antibody. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemias and lymphoid malignancies; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The term "therapeutically effective amount" is used to refer to an amount having antiproliferative effect. Preferably, the therapeutically effective amount has apoptotic activity, or is capable of inducing cell death, and preferably death of benign or malignant tumor cells, in particular cancer cells. 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 cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include 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, 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. I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially an ErbB2-overexpressing cancer cell either in vitro or in vivo. Thus, the growth inhibitory agent is one which significantly reduces the percentage of ErbB2 overexpressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), TAXOL®, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders:

Philadelphia, 1995), especially p. 13. The 4D5 antibody (and functional equivalents thereof) can also be employed for this purpose.

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

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

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

By "solid phase" is meant a non-aqueous matrix to which the antibodies used in accordance with the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g., an 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 antibodies disclosed herein and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

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

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 "peak serum concentration" refers to the maximal 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 drug 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, preferable 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 in 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.

Published information related to anti-ErbB2 antibodies includes the following issued patents and published applications: PCT/US89/00051, published Jan. 5, 1989; PCT/US90/02697, published May 18, 1990; EU 0474727 issued Jul. 23, 1997; DE 69031120.6, issued Jul. 23, 1997; PCT/US97/18385, published Oct. 9, 1997; SA 97/9185, issued Oct. 14, 1997; U.S. Pat. No. 5,677,171, issued Oct. 14, 1997; U.S. Pat. No. 5,720,937, issued Feb. 24, 1998; U.S. Pat. No. 5,720,954, issued Feb. 24, 1998; U.S. Pat. No. 5,725,856, issued Mar. 10, 1998; U.S. Pat. No. 5,770,195, issued Jun. 23, 1998; U.S. Pat. No. 5,772,997, issued Jun. 30, 1998; PCT/US98/2626, published Dec. 10, 1998; and PCT/US99/06673, published Mar. 26, 1999, each of which patents and publications is herein incorporated by reference in its entirety.

II. Production of Anti-ErbB2 Antibodies

A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention. The ErbB2 antigen to be used for production of antibodies may be, e.g., a soluble form of the extracellular domain of ErbB2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing ErbB2 at their cell surface (e.g. NIH-3T3 cells transformed to overexpress ErbB2; or a carcinoma cell line such as SKBR3 cells, see Stancovski et al., *PNAS* (*USA*) 88:8691-8695 [1991]) can be used to generate antibodies. Other forms of ErbB2 useful for generating antibodies will be apparent to those skilled in the art.

(i) Polyclonal Antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

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

(ii) Monoclonal Antibodies

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

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

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

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture

medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

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

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

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

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

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

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

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

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

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

(iii) Humanized and Human Antibodies

Methods for humanizing non-human antibodies are well known in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature,* 321:522-525 (1986); Riechmann et al., *Nature,* 332:323-327 (1988); Verhoeyen et al., *Science,* 239:1534-1536 [1988]), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some 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. Immnol.,* 151:2623 [1993]).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer

programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA,* 90:2551 (1993); Jakobovits et al., *Nature,* 362:255-258 (1993); Bruggermann et al., *Year in Immuno.,* 7:33 (1993). Human antibodies can also be derived from phage-display libraries (Hoogenboom et al., *J. Mol. Biol.,* 227:381 (1991); Marks et al., *J. Mol. Biol.,* 222:581-597 [1991]).

(iv) Antibody Fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan et al., *Science,* 229:81 [1985]). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 [1992]). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185.

(v) Bispecific Antibodies

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the ErbB2 protein. For example, one arm may bind an epitope in Domain 1 of ErbB2 such as the 7C2/7F3 epitope, the other may bind a different ErbB2 epitope, e.g. the 4D5 epitope. Other such antibodies may combine an ErbB2 binding site with binding site(s) for EGFR, ErbB3 and/or ErbB4. Alternatively, an anti-ErbB2 arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the ErbB2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express ErbB2. These antibodies possess an ErbB2-binding arm and an arm which binds the cytotoxic agent (e.g. saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

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

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

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

According to another approach described in WO96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. 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. Shalaby et al., *J. Exp. Med.,* 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

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

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

(vi) Screening for Antibodies with the Desired Properties

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

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

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

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

To 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 can be performed. According to this assay, SKBR3 cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine

serum, glutamine and penicillinstreptomycin. The SKBR3 cells are plated at 20,000 cells in a 35 mm cell culture dish (2 mls/35 mm dish). 2.5 μg/ml of the anti-ErbB2 antibody is added per dish. After six days, the number of cells, compared to untreated cells are counted using an electronic COULTER™ cell counter. Those antibodies which inhibit growth of the SKBR3 cells by 50-100% are selected for combination with the apoptotic antibodies as desired.

(vii) Effector Function Engineering

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

(viii) Immunoconjugates

The invention also pertains to immunoconjugates comprising the antibody described herein conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g. an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated anti-ErbB2 antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al. *Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 94/11026.

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

(ix) Immunoliposomes

The anti-ErbB2 antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., *Proc. Natl. Acad. Sci. USA,* 82:3688 (1985); Hwang et al., *Proc. Natl. Acad. Sci. USA,* 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. 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). 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 covert it into its more active, cytotoxic form.

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

The enzymes of this invention can be covalently bound to the anti-ErbB2 antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins

comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g., Neuberger et al., *Nature,* 312: 604-608 [1984]).

(xi) Antibody-Salvage Receptor Binding Epitope Fusions

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

A systematic method for preparing such an antibody variant having an increased in vivo half-life comprises several steps. The first involves identifying the sequence and conformation of a salvage receptor binding epitope of an Fc region of an IgG molecule. Once this epitope is identified, the sequence of the antibody of interest is modified to include the sequence and conformation of the identified binding epitope. After the sequence is mutated, the antibody variant is tested to see if it has a longer in vivo half-life than that of the original antibody. If the antibody variant does not have a longer in vivo half-life upon testing, its sequence is further altered to include the sequence and conformation of the identified binding epitope. The altered antibody is tested for longer in vivo half-life, and this process is continued until a molecule is obtained that exhibits a longer in vivo half-life.

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

The epitope preferably constitutes a region wherein any one or more amino acid residues from one or two loops of a Fc domain are transferred to an analogous position of the antibody fragment. Even more preferably, three or more residues from one or two loops of the Fc domain are transferred. Still more preferred, the epitope is taken from the CH2 domain of the Fc region (e.g., of an IgG) and transferred to the CH1, CH3, or V_H region, or more than one such region, of the antibody. Alternatively, the epitope is taken from the CH2 domain of the Fc region and transferred to the C_L region or V_L region, or both, of the antibody fragment.

In one most preferred embodiment, the salvage receptor binding epitope comprises the sequence (5' to 3'): PKNSSMISNTP (SEQ ID NO:3), and optionally further comprises a sequence selected from the group consisting of HQSLGTQ (SEQ ID NO:4), HQNLSDGK (SEQ ID NO:5), HQNISDGK (SEQ ID NO:6), or VISSHLGQ (SEQ ID NO:7), particularly where the antibody fragment is a Fab or $F(ab)_2$. In another most preferred embodiment, the salvage receptor binding epitope is a polypeptide containing the sequence(s) (5' to 3'): HQNLSDGK (SEQ ID NO:5), HQNISDGK (SEQ ID NO:6), or VISSHLGQ (SEQ ID NO:7) and the sequence: PKNSSMISNTP (SEQ ID NO:3).

(xii) 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 filter, for example, an 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 antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite 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 C_H3 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 SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following 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 quantitate the amount of specific rhuMAb HER2 (humanized anti-$p185^{HER2}$ 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-$p185^{HER2}$ 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 dilu-

tions 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 $p185^{HER2}$ (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 are washed again with water as in previous steps.

Horse radish peroxidase-conjugate (HRP-conjugate, Goat anti-human IgG Fc 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_2O_2 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 parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Preferred lyophilized anti-ErbB2 antibody formulations are described in WO 97/04801, expressly incorporated herein be 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-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

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

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(−)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C., resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S—S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

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 blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The antibodies of the invention are administered to a human patient, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous 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 antracycline) is desired, the combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. 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 μg/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 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. 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 subcutaneous 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 instructions for use, including, e.g., a warning that the composition is not to be used in combination with anthacycline-type chemotherapeutic agent, e.g. doxorubicin or epirubicin.

Deposit of Materials

The following hybridoma cell lines have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md., USA (ATCC):

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

Further details of the invention are illustrated by the following non-limiting Examples.

EXAMPLES

Example 1: Preparation and Efficacy of HERCEPTIN® Anti-ErbB2 Antibody

Materials and Methods

Anti-ErbB2 Monoclonal Antibody

The anti-ErbB2 $IgG_1\kappa$ murine monoclonal antibody 4D5, specific for the extracellular domain of ErbB2, was produced as described in Fendly et al., *Cancer Research* 5o:1550-1558 (1990) and WO89/06692. Briefly, NIH 3T3/HER2-3_{400} cells (expressing approximately 1×10^5 ErbB2 molecules/cell) produced as described in Hudziak, et al., *Proc. Natl. Acad. Sci. (USA)* 84:7159 (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 ml of the ErbB2 preparation and the splenocytes were fused with mouse myeloma line X63-Ag8.653. Hybridoma supernatants were screened for ErbB2-binding by ELISA and radioimmunoprecipitation. MOPC-21 (IgG1), (Cappell, Durham, N.C.), was used as an isotype-matched control.

The treatment was performed with a humanized version of the murine 4D5 antibody (HERCEPTIN® anti-ErbB2 antibody). The humanized antibody was engineered by inserting the complementarity determining regions of the murine 4D5 antibody into the framework of a consensus human immunoglobulin IgG_1 (IgG_1) (Carter et al., *Proc. Natl. Acad. Sci. USA* 89:4285-4289 [1992]). The resulting humanized anti-ErbB2 monoclonal antibody has high affinity for $p185^{HER2}$ (Dillohiation constant [K_d]=0.1 nmol/L), markedly inhibits, in vitro and in human xenografts, the growth of breast cancer cells that contain high levels of $p185^{HER2}$, induces antibody-dependent cellular cytotoxicity (ADCC), and has been found clinically active, as a single agent, in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior therapy. HERCEPTIN® anti-ErbB2 antibody is produced by a genetically engineered Chinese Hamster Ovary (CHO) cell line, grown in large scale, that secretes the antibody into the culture medium. The antibody is purified from the CHO culture media using standard chromatographic and filtration methods. Each lot of antibody used in this study was assayed to verify identity, purity, and potency, as well as to meet Food and Drug Administration requirements for sterility and safety.

Eligibility Criteria

Patients had to fulfill all of the following criteria to be eligible for study admission:

- Metastatic breast cancer
- Overexpression of the ErbB2 (HER2) oncogene (2+ to 3+ as determined by immunohistochemistry or fluorescence in situ hybridization (FISH). [Tumor expression of ErbB2 can be determined by immunohistochemical analysis, as previously described (Slamon et al., [1987] and [1989], supra), of a set of thin sections prepared from the patient's paraffin-archived tumor blocks. The primary detecting antibody used is murine 4D5 MAb, which has the same CDRs as the humanized antibody used for the treatment. Tumors are considered to overexpress ErbB2 if at least 25% of tumor cells exhibit characteristic membrane staining for $p185^{HER2}$].
- 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:

- Prior cytotoxic chemotherapy for metastatic breast cancer
- Patients may have received prior hormonal therapy (e.g. tamoxifen) for metastatic disease or cytotoxic therapy in the adjuvant setting.
- Concomitant malignancy that has not been curatively treated
- A performance status of <60% on the Karnofsky scale
- Pregnant or nursing women; women of childbearing potential, unless using effective contraception as determined by the investigator

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)

Use of investigational or unlicensed agents within 30 days prior to study entry

Clinically unstable or untreated metastases to the brain (e.g. requiring radiation therapy)

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

Anti-ErbB2 Antibody

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 (T, 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/m^2) 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/m^2) or epirubicin (75 mg/m^2) 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/m^2 over 3 hours by intravenous administration. All patients receiving paclitaxel were premedicated with dexamethasone (or its equivalent) 20 mg×2, administered orally 12 and 6 hours prior to paclitaxel; diphenhydramine (or its equivalent) 50 mg, iv, administered 30 minutes prior to paclitaxel, and dimetidine (or another H_2 blocker) 300 mg, iv, administered 30 minutes prior to paclitaxel.

Response Criteria

Progressive Disease

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 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 1

HERCEPTIN ® Anti-ErbB2 Antibody Efficacy

	Enrolled	TTP (months)	RR (%)	AE (%)
CRx	234	5.5	36.2	66
CRx + H	235	8.6*	62.00**	69
AC	145	6.5	42.1	71
AC + H	146	9.0	64.9	68
T	89	4.2	25.0	59
T + H	89	7.1	57.3	70

*$p < 0.001$ by log-rank test;
**$p < 0.01$ by X^2 test;
CRx: chemotherapy;
AC: anthracycline/cyclophosphamide treatment;
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 3/4) 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.

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

HERCEPTIN ® Anti-ErbB2 Antibody Trough and Peak Serum Concentrations for the First 8 Weeks of Treatment (μg/ml)

	Dose Number	n	Mean	SD	Minimun	Maximum
Peak	1	195	100.3	35.2	30.7	274.6
Trough		195	25.0	12.7	0.16	60.7
Peak	2	190	74.3	31.3	20.8	307.9
Trough		167	30.4	16.0	0.2	74.4
Peak	3	167	75.3	26.8	16.1	194.8
Trough		179	33.7	17.9	0.2	98.2
Peak	4	175	80.2	26.9	22.2	167
Trough		132	38.6	20.1	0.2	89.4
Peak	5	128	85.9	29.2	27.8	185.8
Trough		141	42.1	24.8	0.2	148.7
Peak	6	137	87.2	32.2	28.9	218.1
Trough		115	43.2	24.0	0.2	109.9
Peak	7	114	89.7	32.5	16.3	187.8
Trough		137	48.8	24.9	0.2	105.2
Peak	8	133	95.6	35.9	11.4	295.6

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 (n=40, 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=12.6). 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 8 mg/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 overexpresses the HER2 gene (BT-474M1, 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 3×10^6 BT474M 1 cells suspended in Matrigel™. When tumor nodules reached a volume of approximately 100 mm^3, animals were randomized to 4 treatment groups. The groups were treated according to Table 3.

TABLE 3

Animal Groups and Doses for Comparison of I.V. Bolus and S.C. Infusion

Group, Dose, Antibody	Target Serum Conc. µg/ml	Route of Administration	Loading Dose (mg/kg)	Maintenance Dose
1—Control, rhuMAb E25	20	IV LD and SC infusion	2.20	0.250 mg/ml (infusate)
2—Low Dose SC rhuMAb HER2	1	IV LD and SC infusion	0.313	0.050 mg/ml (infusate)
3—High Dose SC rhuMAb HER2	20	IV LD and SC infusion	6.25	1.00 mg/ml (infusate)
4—IV Multi-Dose rhuMAb HER2	20 (trough)	IV LD and MD	4.00	2 mg/kg/week (IV bolus)

Serum Conc. = concentration in serum.

LD = loading dose.

MD = maintenance dose.

Infusate concentration was calculated to achieve targeted serum concentration using Alzet ® osmotic minipumps (Alza Corp., Palo Alto, CA).

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 BT474M1 cells 8 days before the beginning of treatment and tumors were allowed to grow. The animals were then treated with nonrelevant 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 (just 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 6 to day 35 in the study for which data is tabulated below. Tumors were measured in three dimensions and volumes were expressed in mm^3. 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 BT474M1 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.

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 4

Comparison of S.C. Infusion and I.V. Bolus Delivery

Treatment Group	Tumor Volume (mm^3), Day 35, (n = 14)	Tumor Volume (area under curve) Day 6-Day 35 (n = 13)	HERCEPTIN ® Serum Conc. (µg/ml), Day 27, (n = 3)
control s.c. infusion	764 ± 700	5650 ± 4700	4.16 ± 1.94
s.c. infusion (low dose)	80.6 ± 158	1610 ± 1250	2.11 ± 1.74
s.c. infusion (high dose)	31 ± 75.6	1440 ± 1140	22.1 ± 5.43
i.v. bolus dose*	49.7 ± 95.7	2150 ± 1480	21.7 ± 17.1**

s.c. = subcutaneous delivery;

i.v. = intravenous delivery.

*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)

TABLE 5

Animal Groups and Doses for Comparison of I.V. Bolus and S.C. Bolus Delivery

Group	Route of Administration	Loading Dose (mg/kg)	Maintenance Dose (mg/kg/week)	n
1—Control rhuMAb E25	IV	8	4	10
2—rhuMAb HER2	IV	2	1	10
3—rhuMAb HER2	IV	4	2	10
4—rhuMAb HER2	IV	8	4	10
5—rhuMAb HER2	SC	4	2	10

IV = intraveneous;
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

IV versus SC Bolus Delivery: Serum HERCEPTIN ® Anti-ErbB2 Antibody Concentration
Serum Concentration, μg/ml

Treatment Group (delivery, MD)	Day 0 Mean (SD)	Day 7 Mean (SD)	Day 14 Mean (SD)	Day 21 Mean (SD)
1—Control rhu MAb E25 (IV, 4 mg/kg)	0 (0)	25.9 (8.29)	34.6 (11.2)	38.5 (14.4)
2—rhu MAb HER2 (IV, 1 mg/kg)	0 (0)	4.96 (3.79)	8.55 (5.83)	8.05 (4.67)
3—rhu MAb HER2 (IV, 2 mg/kg)	0 (0)	13.4 (9.24)	18.9 (12.0)	22.6 (9.21)

TABLE 6-continued

IV versus SC Bolus Delivery: Serum HERCEPTIN ® Anti-ErbB2 Antibody Concentration
Serum Concentration, μg/ml

Treatment Group (delivery, MD)	Day 0 Mean (SD)	Day 7 Mean (SD)	Day 14 Mean (SD)	Day 21 Mean (SD)
4—rhu MAb HER2 (IV, 4 mg/kg)	0 (0)	29.6 (13.5)	37.7 (14.4)	46.2 (13.8)
5—rhu MAb HER2 (SC, 2 mg/kg)	0 (0)	12.5 (7.33)	16.9 (10.2)	17.6 (10.7)

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.

TABLE 7

Efficacy of HERCEPTIN ® Anti-ErbB2 Antibody Measured as a Change in Tumor Volume Comparing Intravenous Bolus and Subcutaneous Bolus Delivery, Mean (SD)

Treatment Group (Delivery, MD)	Tumor Vol. Day 6, mm^3	Tumor Vol. Day 28, mm^3	Tumor Vol. Day 31, mm^3	Day 6-Day 31* Area Under Curve Tumor Vol., mm^3	Tumor Growth Rate on Log (TM + 1)
1—IV Control	321 (190)	1530 (1040)	1630 (1170)	13600 (7230)	0.0660 (0.0200)
2—IV Herceptin 1 mg/kg	297 (130)	175 (215)	151 (188)	4690 (1400)	−0.0505 (0.142)
3—IV Herceptin 2 mg/kg	269 (129)	75.7 (92.4)	73.6 (84.5)	3510 (1220)	−0.0608 (0.110)
4—IV Herceptin 4 mg/kg	272 (117)	25.3 (75.9)	25.8 (72.9)	2880 (1230)	−0.0810 (0.0859)
5—SC Herceptin 2 mg/kg	268 (117)	76.2 (98.8)	90.4 (105)	3230 (1440)	−0.0304 (0.104)

N = 10 for each data point.
TM = tumor measurement.
IV = intravenous.
SC = subcutaneous.
MD = maintenance dose.
Tumor Vol. = tumor volume, mm^3.
*Day 17 excluded due to measurement error.
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.

FIGS. **4**A and **4**B are graphical plots of changes in tumor volume over time, some of which data is found in Table 7. FIG. **4**A is a linear plot of tumor volume versus time. FIG. **4**B is a semilogarithmic plot of the same data, allowing the test points be viewed more clearly. The data in Table 7 and FIGS. **4**A and **4**B indicate that, although a dose-related response was not observed between HERCEPTIN-treated groups, dosing by subcutaneous bolus was as effective as intravenous bolus dosing and achieved similar trough serum concentrations.

Example 5: Regimens for Intravenous and Subcutaneous Delivery of Anti-ErbB2 Antibody

According to the invention, methods of anti-ErbB2 antibody (e.g., HERCEPTIN®) delivery comprise greater front loading of the drug to achieve a 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 inven-

tion, this initial dosing is followed by dosing that maintains the target serum concentration by subsequent doses of equal or smaller amount. An advantage of the methods of the invention is that the maintenance dosing may be less frequent and/or delivered by subcutaneous injection, making the treatment regimens of the invention convenient and cost-effective for the patient and medical professionals administering the antibody. In addition, a subcutaneous maintenance dose regimen may be interrupted by intravenous dosing (such as infusion) when the patient's chemotherapy requires delivery of other drugs by intravenous injection.

To test the following dosage regimens, human subjects are selected according to the criteria disclosed in Example 1, above. The number of initial doses is one or more doses 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 1 day or less. The number of maintenance doses may be one or more doses sufficient to achieve suppression of disease symptoms, such as a decrease in tumor volume. The maintenance doses are 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/m^1 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 delivery subsequent maintenance doses.

In another method, an initial (front loading) dose of 8 mg/kg HERCEPTIN® anti-ErbB2 antibody is delivered by intravenous injection (infusion 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 injection (infusion 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 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 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 example, 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 is optionally and preferably deliverable 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 anthrocycline 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 175 mg/m2 every 3 weeks) or an anthracy-

cline 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/m^2/week. According to another embodiment of the invention, the taxoid is docetaxel and is administered at a dose of 30-70 mg/m^2/week.

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/m^2 every three weeks). Simulation of the proposed treatment regimen suggests that the trough serum concentrations will be 17 mcg/ml, in the range (10-20 mcg/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 spermicidal 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 3.0×10^9/l
   Granulocytes less than 1.5×10^9/l
   Platelets less than 100×10^9/l
8) Any of the following abnormal baseline liver function tests:
   Serum bilirubin greater than 1.5×ULN (upper normal limit)
   ALT and/or AST greater than 2.5×ULN (greater than 4.0×ULN if liver or bone metastasis)
   Alkaline phosphatase greater than 2.5×ULN (greater than 4.0×ULN if liver or bone metastasis)
9) The following abnormal baseline renal function tests:
   serum creatinine greater than 1.5×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/m^2 IV every 3 weeks×6 cycles as a 3-hour infusion.

NOTE: On the first cycle of treatment, paclitaxel will be dosed 8 hours prior to HERCEPTIN® to determine the PK of paclitaxel alone. HERCEPTIN® will be administered 8 hours post-paclitaxel for the 1st cycle only. In subsequent treatment cycles, HERCEPTIN® will be administered prior to paclitaxel.

The total duration of this study is 18 weeks. Study subjects will receive up to 6 total HERCEPTIN® doses. After the last subject has received the last cycle of paclitaxel, data collection for safety and pharmacokinetic analysis will stop, and the study will close to protocol specified treatment. Study subjects may continue to receive the HERCEPTIN®+/−paclitaxel at the discretion of the investigator.

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.

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35 40 45

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Thr Leu Val Thr Val Ser Ser
115

The invention claimed is:

1. A method for the treatment of a human patient diagnosed with breast cancer characterized by 2+ or 3+ overexpression of ErbB2 receptor as determined by immunohistochemistry or fluorescence in situ hybridization (FISH), comprising the steps of administering to the patient an initial dose of 8 mg/kg of anti-ErbB2 huMAb 4D5-8 antibody; and administering to the patient a plurality of subsequent doses of 6 mg/kg of the antibody, wherein all doses are separated in time from each other by three weeks.

2. The method of claim **1**, further comprising administering an effective amount of a chemotherapeutic agent.

3. The method of claim **2**, wherein said chemotherapeutic agent is a taxoid.

4. The method of claim **3**, wherein said taxoid is paclitaxel or docetaxel.

5. The method of claim **4** wherein said taxoid is paclitaxel.

6. The method of claim **1**, wherein said antibody is administered by intravenous injection.

7. A method for the treatment of a human patient diagnosed with breast cancer characterized by 2+ or 3+ overexpression of ErbB2 receptor as determined by immunohistochemistry or fluorescence in situ hybridization (FISH), the method comprising: administering intravenously to the patient an initial dose of 8 mg/kg of anti-ErbB2 huMAb 4D5-8 antibody; and administering intravenously to the patient a plurality of subsequent 6 mg/kg doses of the antibody, wherein the initial dose is separated in time from the first subsequent dose by three weeks, and the subsequent doses are separated from each other in time by three weeks.

8. The method of claim **7**, wherein the intravenous administration is an intravenous infusion.

9. The method of claim **8**, wherein the subsequent doses maintain a trough serum concentration of the anti-ErbB2 huMAb 4D5-8 antibody at or above 10 μg/mL.

10. The method of claim **8**, wherein the subsequent doses maintain a trough serum concentration of the anti-ErbB2 huMAb 4D5-8 antibody at or above 20 μg/mL.

11. The method of claim **7**, wherein the subsequent doses maintain a trough serum concentration of the anti-ErbB2 huMAb 4D5-8 antibody at or above 10 μg/mL.

12. The method of claim **7**, wherein the subsequent doses maintain a trough serum concentration of the anti-ErbB2 huMAb 4D5-8 antibody at or above 20 μg/mL.

* * * * *

CERTIFICATE OF SERVICE

I hereby certify that, on this 26th day of July, 2019, I filed the foregoing Non-Confidential Brief for Plaintiffs-Appellants Genentech Inc. with the Clerk of the United States Court of Appeals for the Federal Circuit via the CM/ECF system, which will send notice of such filing to all registered CM/ECF users.

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CERTIFICATE OF COMPLIANCE

Pursuant to Fed. R. App. P. 32(g), the undersigned hereby certifies that this brief complies with the type-volume limitation of Federal Circuit Rule 32(a).

1. Exclusive of the exempted portions of the brief, as provided in Fed. R. App. P. 32(f) and Fed. Cir. R. 32(b), the brief contains 13,006 words.

2. The brief has been prepared in proportionally spaced typeface using Microsoft Word 2010 in 14 point Times New Roman font. As permitted by Fed. R. App. P. 32(g), the undersigned has relied upon the word count feature of this word processing system in preparing this certificate.

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