HU000035605T2

(19) **HU**

(11) Lajstromszám: **E 035 605** (13) **T2**

MAGYARORSZÁG
Szellemi Tulajdon Nemzeti Hivatala

EURÓPAI SZABADALOM
SZÖVEGÉNEK FORDÍTÁSA

(21) Magyar ügyszám: **E 13 189092**
(22) A bejelentés napja: **2010. 11. 12.**

(96) Az európai bejelentés bejelentési száma:
EP 20100189092
(97) Az európai bejelentés közzétételi adatai:
EP 2719708 A2 **2014. 04. 16.**
(97) Az európai szabadalom megadásának meghirdetési adatai:
EP 2719708 B1 **2017. 10. 25.**

(51) Int. Cl.: ***C07K 16/28*** *(2006.01)*
C07K 16/32 *(2006.01)*
C07K 16/30 *(2006.01)*

(30) Elsőbbségi adatok:
261149 P **2009. 11. 13.** **US**

(72) Feltaláló(k):
Hettmann, Thore, 9051 Sint-Denijs-Westrem (BE)
Freeman, Daniel J., Newbury Park, CA 91320 (US)
RADINSKY, Robert, Thousand Oaks, CA 91362 (US)

(73) Jogosult(ak):
Daiichi Sankyo Europe GmbH, 81379 München (DE)
Amgen Inc., Thousand Oaks, California 91320-1799 (US)

(74) Képviselő:
Advopatent Szabadalmi és Védjegy Iroda, Budapest

(54) **Anyag és eljárások HER-3-mal összefüggő betegségek kezelésére vagy megelőzésére**

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 719 708 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
25.10.2017 Bulletin 2017/43

(21) Application number: **13189092.3**

(22) Date of filing: **12.11.2010**

(51) Int Cl.:
C07K 16/28 *(2006.01)* ***C07K 16/30*** *(2006.01)*
C07K 16/32 *(2006.01)*

(54) **Material and methods for treating or preventing HER-3 associated diseases**

Material und Verfahren zur Behandlung oder Vorbeugung von HER-3-assoziierten Erkrankungen

Matériau et procédés de traitement ou de prévention de maladies associées à des HER-3

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **13.11.2009 US 261149 P**

(43) Date of publication of application:
16.04.2014 Bulletin 2014/16

(60) Divisional application:
15154165.3 / 2 896 632

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
10785251.9 / 2 499 162

(73) Proprietors:
- **Daiichi Sankyo Europe GmbH**
  81379 München (DE)
- **Amgen Inc.**
  Thousand Oaks, California 91320-1799 (US)

(72) Inventors:
- **Hettmann, Thore**
  9051 Sint-Denijs-Westrem (BE)
- **Freeman, Daniel J.**
  Newbury Park, CA 91320 (US)
- **Radinsky, Robert**
  Thousand Oaks, CA 91362 (US)

(74) Representative: **Weickmann & Weickmann PartmbB**
Postfach 860 820
81635 München (DE)

(56) References cited:
WO-A2-2007/077028 WO-A2-2008/100624
WO-A2-2011/022727

- **TREDER M ET AL: "309 POSTER Fully human anti-HER3 mAb U3-1287 (AMG 888) demonstrates unique in vitro and in vivo activities versus other HER family inhibitors in NSCLC models", EUROPEAN JOURNAL OF CANCER. SUPPLEMENT, PERGAMON, OXFORD, GB, vol. 6, no. 12, 1 October 2008 (2008-10-01), page 99, XP025534373, ISSN: 1359-6349, DOI: DOI:10.1016/S1359-6349(08)72243-2 [retrieved on 2008-10-01]**
- **HSIEH A C ET AL: "Targeting HER proteins in cancer therapy and the role of the non-target HER3", BRITISH JOURNAL OF CANCER, NATURE PUBLISHING GROUP, LONDON, GB, vol. 97, no. 4, 1 August 2007 (2007-08-01) , pages 453-457, XP009123247, ISSN: 0007-0920**
- **Merrimack Pharmaceuticals: "A study of MM-121 combination therapy in patients with advanced non-small cell lung cancer", ClinicalTrials.gov , 13 October 2009 (2009-10-13), XP002726209, Retrieved from the Internet: URL:http://www.clinicaltrials.gov/ct2/show /record/NCT00994123?term=MM-121&rank=1 [retrieved on 2014-06-25] -& "View of NCT00994123 on 2009_10_13", ClinicalTrials.gov archive , 13 October 2009 (2009-10-13), XP002726210, Retrieved from the Internet: URL:http://clinicaltrials.gov/archive/NCT0 0994123/2009_10_13 [retrieved on 2014-06-25]**

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

- **JONES P T ET AL: "REPLACING THE COMPLEMENTARITY-DETERMINING REGIONS IN A HUMAN ANTIBODY WITH THOSE FROM A MOUSE", NATURE, NATURE PUBLISHING GROUP, UNITED KINGDOM, vol. 321, 29 May 1986 (1986-05-29), pages 522-525, XP002949266, ISSN: 0028-0836, DOI: 10.1038/321522A0**
- **OHNO S ET AL: "Antigen-binding specificities of antibodies are primarily determined by seven residues of VH.", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA MAY 1985, vol. 82, no. 9, May 1985 (1985-05), pages 2945-2949, ISSN: 0027-8424**

Description

BACKGROUND

1. Technical Field

[0001] This document relates to materials and methods for treating subjects having a hyperproliferative disease associated with Human Epidermal Growth Factor Receptor -3 (HER-3) by administering a first agent that binds to HER-3, in combination with trastuzumab.

2. Background

[0002] HER-3, also known as ErbB3, is a receptor protein tyrosine kinase that belongs to the epidermal growth factor receptor (EGF-R, also known as HER) family of receptor protein tyrosine kinases, which also includes HER-1 (also known as EGF-R or erbB), HER-2 (also known as erbB2), and HER-4 (also known as erbB4) (Plowman et al. (1990) Proc. Natl. Acad. Sci. US 87:4905-4909; Kraus et al. (1989) Proc. Natl. Acad. Sci. US 86:9193-9197; and Kraus et al. (1993) Proc. Natl. Acad. Sci. US 90:2900-2904). Like the prototypical epidermal growth factor receptor, the transmembrane receptor HER-3 consists of an extracellular ligand-binding domain (ECD), a dimerization domain within the ECD, a transmembrane domain (TMD), an intracellular protein tyrosine kinase domain (TKD), and a C-terminal phosphorylation domain.

[0003] The ligand for HER-3, known as heregulin (HRG), binds to the extracellular domain of HER-3 and activates receptor-mediated signaling by promoting dimerization with other human epidermal growth factor receptor (HER) family members, subsequent transphosphorylation of the intracellular HER-3 domain, and activation of downstream signaling cascades. Dimer formation with multiple HER family members expand the signaling potential of HER-3, and is a means for signal diversification as well as signal amplification.

[0004] WO 2007/077028 relates to antibodies directed to HER-3 and uses thereof. Particular antibodies that bind to HER-3 and polynucleotides encoding the same are disclosed. Also described are expression vectors and host cells comprising the same for the production of the disclosed antibodies.

SUMMARY

[0005] This document relates to a first agent and a second agent for use in the treatment or prevention of a hyperproliferative disease associated with HER-3, wherein said hyperproliferative disease is HER-3 expressing or overexpressing cancer, wherein said second agent is trastuzumab, and
wherein said first agent is an antigen-binding protein that binds to HER-3, and comprises: a heavy chain amino acid sequence that comprises a CDRH1 as shown in SEQ ID NO: 256; a CDRH2 as shown in SEQ ID NO: 282; and a CDRH3 as shown in SEQ ID NO: 315; and a light chain amino acid sequence that comprises a CDRL1 as shown in SEQ ID NO: 340; a CDRL2 as shown in SEQ ID NO: 344; and a CDRL3 as shown in SEQ ID NO: 387.

[0006] Other HER3-binding proteins are provided as comparative examples only. According to the invention, the first agent can be an antigen-binding protein that binds to HER-3 and comprises a heavy chain amino acid sequence as shown in SEQ ID NO: 70. The antigen-binding protein can include a light chain amino acid sequence as shown in SEQ ID NO: 72.

[0007] The antigen-binding protein can be directed against the extracellular domain of HER-3. Binding of the antigen-binding protein to HER-3 can reduce HER-3-mediated signal transduction, reduce HER-3 phosphorylation, reduce cell proliferation, reduce cell migration, and/or increase the downregulation of HER-3.

[0008] The antigen-binding protein that binds to HER-3 can be an antibody. The antibody can be a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a humanized antibody, a human antibody, a chimeric antibody, a multi-specific antibody, or an antibody fragment thereof (e.g., a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fv fragment, a diabody, or a single chain antibody molecule). The antibody can be of the IgG1-, IgG2-, IgG3- or IgG4-type.

[0009] The first agent can be an antigen-binding protein that binds to HER-3, and the antigen-binding protein can be coupled to an effector group. The effector group can be a radioisotope or radionuclide, a toxin, or a therapeutic or chemotherapeutic group (e.g., a therapeutic or chemotherapeutic group selected from the group consisting of calicheamicin, auristatin-PE, geldanamycin, maytansine and derivatives thereof).

[0010] The second agent is trastuzumab. Other agents are provided as comparative examples.

[0011] The methods provided herein can optionally include administering a third or further therapeutic agent and/or radiation therapy. The third or further therapeutic agent can be an anti-neoplastic agent (*e.g.*, an anti-tumor antibody or a chemotherapeutic agent, such as capecitabine, anthracycline, doxorubicin, cyclophosphamide, paclitaxel, docetaxel, cisplatin, gemcitabine, or carboplatin).

[0012] The first agent and the second agent can be administered by intravenous, subcutaneous, intramuscular or oral administration. According to the invention, the disease is HER3 expressing or overexpressing cancer (*e.g.*, selected from the group consisting of breast cancer, ovarian cancer, prostate cancer, colon cancer, renal cancer, lung cancer, pancreatic cancer, epidermoid carcinoma, fibrosarcoma, melanoma, nasopharyngeal carcinoma, and squamous cell carcinoma).

[0013] The methods described herein can include administering the first agent at a dose of about 1 to about 20 mg/kg body weight, at least once every 6 weeks. The methods can include administering the second agent at a dose of about 1 to about 20 mg/kg body weight, at least once every 6 weeks. The methods can further include, prior to the administering, using a method that comprises analysis of a predictive marker to select a subject having a disease associated with HER-3. The methods can further include after the administering, monitoring the therapeutic outcome.

[0014] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0015] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016]

FIG. 1 is a graph plotting the effects of a human anti-HER-3 antibody and panitumumab, either alone or in combination, on non-small cell lung cancer (NSCLC) xenograft tumor (Calu-3) growth.

FIG. 2 is a graph plotting the effects of a human anti-HER-3 antibody and erlotinib, either alone or in combination, on Calu-3 growth.

FIG. 3 is a graph plotting the effects of a human anti-HER-3 antibody, either alone or in combination with c2C4 (a HER2 dimerization inhibitor), or trastuzumab on basal anchorage-independent growth of SkBr-3 breast cancer cells.

FIG. 4 is a graph plotting the effects of a human anti-HER-3 antibody, either alone or in combination with c2C4, trastuzumab, or cetuximab, on HRG stimulated anchorage-independent growth of SkBr-3 breast cancer cells.

FIG. 5 is a graph plotting the effects of a human anti-HER-3 antibody, either alone or in combination with 2C4), trastuzumab, or cetuximab, on basal anchorage-independent growth of MDA-MB-435 ovarian cancer cells.

FIGS. 6A-6D are a series of graphs plotting the effects of a human anti-HER-3 antibody, either alone or in combination with trastuzumab (FIG. 6A), lapatinib (FIG. 6B), gemcitibine (FIG. 6C), or cisplatin (FIG. 6D), on proliferation of MDA-MB-175VII breast cancer cells.

FIG. 7 is a graph plotting the effects of a human anti-HER-3 antibody, either alone or in combination with c2C4, trastuzumab, or lapatinib, on HRG stimulated proliferation of ZR-75-30 breast cancer cells.

FIG. 8 is a graph plotting the effects of a human anti-HER-3 antibody, either alone or in combination with c2C4, trastuzumab, or lapatinib, on HRG stimulated proliferation of BT474 breast cancer cells.

FIG. 9 is a graph plotting the effects of a human anti-HER-3 antibody, either alone or in combination with cetuximab, c2C4, or trastuzumab, on proliferation of HRG stimulated DLD-1 colon cancer cells.

FIG. 10 is a graph plotting the effects of a human anti-HER-3 antibody, either alone or in combination with c2C4, or trastuzumab, or lapatinib on HRG stimulated proliferation of HCC-1569 breast cancer cells.

FIG. 11 is a graph plotting the effects of a human anti-HER-3 antibody, either alone or in combination with 2C4, trastuzumab, or lapatinib, on HRG stimulated proliferation of SkBr-3 breast cancer cells.

FIG. 12 is a graph plotting the effects of a human anti-HER-3 antibody, either alone or in combination with panitumumab on proliferation of FaDu head and neck cancer cells.

FIG. 13 is a picture of a Western blot showing the effects of a human anti-HER-3 antibody, either alone or in combination with cetuximab, c2C4, or trastuzumab, on phosphorylation of HER-3 (top panel), Akt (middle panel), and ERK (bottom panel) in MDA-MB-175VII breast cancer cells.

FIG. 14 is a picture of a Western blot showing the effects of a human anti-HER-3 antibody, either alone or in combination with cetuximab, c2C4, trastuzumab, or lapatinib, on phosphorylation of HER-3 (top panel), Akt (middle panel), and ERK (bottom panel) in HRG stimulated SkBr-3 breast cancer cells.

FIG. 15 is a picture of a Western blot showing the effects of a human anti-HER-3 antibody, either alone or in combination with cetuximab, pertuzumab (c2C4), or trastuzumab, on phosphorylation of HER-3 (top panel) or Akt (bottom panel) in HRG stimulated Ls174T colon cancer cells.

FIG. 16 is a picture of a Western blot showing the effects of a human anti-HER-3 antibody, either alone or in

combination with cetuximab, c2C4, or trastuzumab, on phosphorylation of HER-3 (top panel), Akt (middle panel), and ERK (bottom panel) in HRG stimulated HCC 1569 breast cancer cells.

FIG. 17 is a picture of a Western blot showing the effects of a human anti-HER-3 antibody, either alone or in combination with panitumumab, on phosphorylation of Akt, PGFR, HER-2, HER-3, HER-4, and ERK in A549 alveolar epithelial cells. Lane 1, IgG control; lane 2, panitumumab, alone; lane 3, U1-59, alone; lane 4, U1-59, in combination with panitumumab. Tubulin was used as a control for equal loading.

FIG. 18 is a picture of a Western blot showing the effects of a human anti-HER-3 antibody, either alone or in combination with panitumumab or lapatinib, on phosphorylation of HER-3, Akt, HER-2, ERK, and EGF-R in Calu3 NSCLC cells. Lane 1, IgG control; lane 2, panitumumab alone; lane 3, U1-59 alone; lane 4, lapatinib alone; lane 5, U1-59 in combination with panitumumab; lane 6, U1-59 in combination with lapatinib.

FIG. 19 is a graph plotting the effects of a human anti-HER-3 antibody and lapatinib, either alone or in combination, on breast cancer xenograft tumor (HCC-1569) growth.

FIG. 20 shows that treatment of A549 NSCLC cells with U1-59 inhibits HER3 phosphorylation and reduces reactivation after treatment with gefitinib. A549 cells were treated with gefitinib, U1-59 or both, and HER3 phosphorylation was evaluated by ELISA analysis. Treatment with gefitinib for 1 hour resulted in partial inhibition of HER phosphorylation, which was reversed to control levels after 24 hours. In contrast, treatment with U1-59 led to greater inhibition of HER phosphorylation that was sustained after 24 hours. Combined treatment with both agents prevented the reversal of inhibition seen after 24 hours in cells treated with gefitinib alone. Experiments were performed in triplicate wells and repeated at least 2 times. Results are expressed as mean ±SD.

DETAILED DESCRIPTION

[0017] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0018] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0019] Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present application are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See, e.g.,* Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001), Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0020] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the disclosed, which is defined solely by the claims.

[0021] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages may mean.+/-,1%.

1. General Overview

[0022] This document describes materials and methods related to treating or preventing diseases associated with HER-3, using a combination of a first agent that binds to HER-3, and a second agent that binds to/or inhibits the activity of other members of the HER family. The subject-matter of the invention is a first agent and a second agent for use in the treatment or prevention of a hyperproliferative disease associated with HER-3, wherein said hyperproliferative disease is HER-3 expressing or overexpressing cancer, wherein said second agent is trastuzumab, and

wherein said first agent is an antigen-binding protein that binds to HER-3, and comprises: a heavy chain amino acid sequence that comprises a CDRH1 as shown in SEQ ID NO: 256; a CDRH2 as shown in SEQ ID NO: 282; and a CDRH3 as shown in SEQ ID NO: 315; and a light chain amino acid sequence that comprises a CDRL1 as shown in SEQ ID NO: 340; a CDRL2 as shown in SEQ ID NO: 344; and a CDRL3 as shown in SEQ ID NO: 387.

2. HER-3 binding agents

[0023] As described herein, the agent that binds to HER-3 is an antigen binding protein, such as an antibody. As used herein, an "antigen binding protein" or "binding protein" as used herein means a protein that specifically binds a specified target antigen, such as member of the HER family, *e.g.*, HER-3. An antigen binding protein is said to "specifically bind" its target antigen when the dissociation constant (K_D) is $\leq 10^{-8}$ M. The antibody specifically binds antigen with "high affinity" when the K_D is $\leq 5x10^{-9}$ M, and with "very high affinity" when the K_D is $\leq 5x\ 10^{-10}$ M. In one embodiment, the antibody has a K_D of $\leq 10^{-9}$ M and an off-rate of about $1x.10^{-4}$/sec. In one embodiment, the off-rate is about $1x.10^{5}$/sec. In other embodiments, the antibodies will bind to a specified member of the HER family with a K_D of between about 10^{-8} M and 10^{-10} M, and in yet another embodiment it will bind with a $K_D \leq 2x.10^{-10}$. An antibody targeted to HER-3 can be directed against the extracellular domain (ECD) of HER-3. For example, an anti-HER-3 antibody as described herein can interact with at least one epitope in the extracellular part of HER-3. The epitopes can be located in the amino terminal L1 domain (aa 19-184), in the S1 (aa 185-327) and S2 (aa 500-632) cysteine-rich domains, in the L2 domain (328-499), which is flanked by the two cysteine-rich domains, or in a combination of HER-3 domains. The epitopes also may be located in combinations of domains such as, without limitation, an epitope comprised by parts of L1 and S1.

[0024] A HER-3 binding protein can be further characterized in that its binding to HER-3 reduces HER-3-mediated signal transduction. A reduction of HER-3-mediated signal transduction may, *e.g.,* be caused by a downregulation of HER-3 resulting in an at least partial disappearance of HER-3 molecules from the cell surface or by a stabilization of HER-3 on the cell surface in a substantially inactive form, *i.e.*, a form that exhibits a lower signal transduction compared to the non-stabilized form. Alternatively, a reduction of HER-3-mediated signal transduction also may be caused by influencing, *e.g.*, decreasing or inhibiting, the binding of a ligand or another member of the HER family to HER-3. For example, a reduction of HER-3 mediated signal transduction also can be caused by, decreasing the formation of HER-3 containing dimers with other HER family members (*e.g.*, EGF-R).

[0025] A HER-3 binding agent can be a scaffold protein having an antibody-like binding activity (*e.g.,* having activity similar to an anti-HER-3 antibody) or an antibody, *i.e.,* an anti-HER-3 antibody. As used herein, the term "scaffold protein" means a polypeptide or protein with exposed surface areas in which amino acid insertions, substitutions or deletions are highly tolerable. Examples of scaffold proteins that can be used in accordance with the present methods include protein A from *Staphylococcus aureus*, the bilin binding protein from *Pieris brassicae* or other lipocalins, ankyrin repeat proteins, and human fibronectin (reviewed in Binz and Plückthun (2005) Curr. Opin. Biotechnol. 16:459-69). Engineering of a scaffold protein can be regarded as grafting or integrating an affinity function onto or into the structural framework of a stably folded protein. Affinity function means a protein binding affinity according to the present document. A scaffold can be structurally separable from the amino acid sequences conferring binding specificity. In general, proteins appearing suitable for the development of such artificial affinity reagents may be obtained by rational, or most commonly, combinatorial protein engineering techniques such as panning against HER-3, either purified protein or protein displayed on the cell surface, for binding agents in an artificial scaffold library displayed *in vitro,* skills which are known in the art (*see, e.g.,* Skerra (2000) J. Mol. Recog. 13:167-87; and Binz and Plückthun, *supra*). In addition, a scaffold protein having an antibody like binding activity can be derived from an acceptor polypeptide containing the scaffold domain, which can be grafted with binding domains of a donor polypeptide to confer the binding specificity of the donor polypeptide onto the scaffold domain containing the acceptor polypeptide. The inserted binding domains may be, for example, the complementarity determining region (CDR) of an antibody, in particular an anti-HER-3 antibody. Insertion can be accomplished by various methods known to those skilled in the art including, for example, polypeptide synthesis, nucleic acid synthesis of an encoding amino acid as well by various forms of recombinant methods well known to those skilled in the art.

[0026] The term "antibody" includes monoclonal antibodies, polyclonal antibodies, recombinant antibodies, humanized antibodies (Jones et al. (1986) Nature 321:522-525; Riechmann et al. (1988) Nature 332:323-329; and Presta (1992) Curr. Op. Struct. Biol. 2:593-596), chimeric antibodies (Morrison et al. (1984) Proc. Natl. Acad. Sci. US 81:6851-6855), multispecific antibodies (*e.g.*, bispecific antibodies) formed from at least two antibodies, or antibody fragments thereof. The term "antibody fragment" comprises any portion of the aforementioned antibodies, such as their antigen binding or variable regions. Examples of antibody fragments include Fab fragments, Fab' fragments, $F(ab')_2$ fragments, Fv fragments, diabodies (Hollinger et al. (1993) Proc. Natl. Acad. Sci. US 90:6444-6448), single chain antibody molecules (Plückthun in: The Pharmacology of Monoclonal Antibodies 113, Rosenburg and Moore, eds., Springer Verlag, NY (1994), 269-315) and other fragments as long as they exhibit the desired capability of binding to HER-3.

[0027] In addition, the term "antibody," as used herein, includes antibody-like molecules that contain engineered subdomains of antibodies or naturally occurring antibody variants. These antibody-like molecules may be single-domain antibodies such as V_H-only or V_L-only domains derived either from natural sources such as camelids (Muyldermans et al. (2001) Rev. Mol. Biotechnol. 74:277-302) or through *in vitro* display of libraries from humans, camelids or other species (Holt et al. (2003) Trends Biotechnol. 21:484-90).

[0028] An "Fv fragment" is the minimum antibody fragment that contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy chain variable domain and one light chain variable domain in tight,

non-covalent association., It is in this configuration that the three CDR's of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDR's confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDR's specific for an antigen) has the ability to recognize and bind the antigen, although usually 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. The "Fab fragment" differs from the "Fab' fragment" 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. The "$F(ab')_2$ fragment" originally is produced as a pair of "Fab' fragments" which have hinge cysteines between them. Methods of preparing such antibody fragments, such as papain or pepsin digestion, are known to those skilled in the art.

[0029] An antibody can be of the IgA-, IgD-, IgE, IgG- or IgM-type, including IgG- or IgM-types such as, without limitation, IgG1-, IgG2-, IgG3-, IgG4-, IgMl- and IgM2-types. For example, in some cases, the antibody is of the IgG1-, IgG2- or IgG4- type.

[0030] In certain respects, *e.g.*, in connection with the generation of antibodies as therapeutic candidates against HER-3, it may be desirable that the antibody is capable of fixing complement and participating in complement-dependent cytotoxicity (CDC). There are a number of isotypes of antibodies that are capable of the same including: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgG1, human IgG3, and human IgA, for example. It will be appreciated that antibodies that are generated need not initially possess such an isotype but, rather the antibody as generated can possess any isotype and the antibody can be isotype switched by appending the molecularly cloned V region genes or cDNA to molecularly cloned constant region genes or cDNAs in appropriate expression vectors using conventional molecular biological techniques that are well known in the art and then expressing the antibodies in host cells using techniques known in the art. The isotype-switched antibody may also possess an Fc region that has been molecularly engineered to possess superior CDC over naturally occurring variants (Idusogie et al. (2001) J. Immunol. 166:2571-2575) and expressed recombinantly in host cells using techniques known in the art. Such techniques include the use of direct recombinant techniques (*see, e.g.,* US Patent No. 4,816,397), cell-cell fusion techniques (*see, e.g.,* US Patent Nos. 5,916,771 and 6,207,418), among others. In the cell-cell fusion technique, a myeloma or other cell line such as CHO is prepared that possesses a heavy chain with any desired isotype and another myeloma or other cell line such as CHO is prepared that possesses the light chain. Such cells can thereafter be fused, and a cell line expressing an intact antibody can be isolated. By way of example, a human anti-HER-3 IgG4 antibody that possesses the desired binding to the HER-3 antigen can be readily isotype switched to generate a human IgM, human IgG1 or human IgG3 isotype, while still possessing the same variable region (which defines the antibody's specificity and some of its affinity). Such a molecule might then be capable of fixing complement and participating in CDC.

[0031] Moreover, an antibody also may be capable of binding to Fc receptors on effector cells such as monocytes and natural killer (NK) cells, and participating in antibody-dependent cellular cytotoxicity (ADCC). There are a number of antibody isotypes that are capable of the same, including, without limitation, the following: murine IgG2a, murine IgG2b, murine IgG3, human IgG1 and human IgG3. It will be appreciated that the antibodies that are generated need not initially possess such an isotype but, rather the antibody as generated can possess any isotype and the antibody can be isotype switched by appending the molecularly cloned V region genes or cDNA to molecularly cloned constant region genes or cDNAs in appropriate expression vectors using conventional molecular biological techniques that are well known in the art and then expressing the antibodies in host cells using techniques known in the art. The isotype-switched antibody may also possess an Fc region that has been molecularly engineered to possess superior ADCC over naturally occurring variants (Shields et al. (2001) J. Biol. Chem. 276:6591-604) and expressed recombinantly in host cells using techniques known in the art. Such techniques include the use of direct recombinant techniques (*see, e.g.,* US Patent No. 4,816,397), cell-cell fusion techniques *(see, e.g.,* US Patent Nos. 5,916,771 and 6,207,418), among others. In the cell-cell fusion technique, a myeloma or other cell line such as CHO is prepared that possesses a heavy chain with any desired isotype and another myeloma or other cell line such as CHO is prepared that possesses the light chain. Such cells can thereafter be fused, and a cell line expressing an intact antibody can be isolated. By way of example, a human anti-HER-3 IgG4 antibody that possesses the desired binding to the HER-3 antigen could be readily isotype switched to generate a human IgG1 or human IgG3 isotype, while still possessing the same variable region (which defines the antibody's specificity and some of its affinity). Such molecule might then be capable of binding to FcγR on effectors cells and participating in ADCC.

[0032] TABLE 10 herein provides amino acid sequences for a number of CDR's that can be included in antibodies against HER-3. An isolated binding protein targeted to HER-3 can include a heavy chain amino acid sequence containing at least one CDR selected from the group consisting of: (a) CDRH1's as shown in SEQ ID NOS:2, 6, 10, 14, 18, 22, 26, 30, 34, 36, 40, 42, 46, 50, 54, 60, 62, 66, 70, 74, 78, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 122, 126, 130, 134, 138, 142, 146, 150, 154, 158, 162, 166, 170, 174, 178, 182, 186, 190, 194, 198, 202, 206, 210, 214, 218, 222, 226 and 230, (b) CDRH2's as shown in SEQ ID NOS:2, 6, 10, 14, 18, 22, 26, 30, 34, 36, 40, 42, 46, 50, 54, 60, 62, 66, 70, 74, 78, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 122, 126, 130, 134, 138, 142, 146, 150, 154, 158, 162, 166, 170, 174, 178, 182, 186, 190, 194, 198, 202, 206, 210, 214, 218, 222, 226 and 230, and (c) CDRH3's as shown in SEQ

ID NOS:2, 6, 10, 14, 18, 22, 26, 30, 34, 36, 40, 42, 46, 50, 54, 60, 62, 66, 70, 74, 78, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 122, 126, 130, 134, 138, 142, 146, 150, 154, 158, 162, 166, 170, 174, 178, 182, 186, 190, 194, 198, 202, 206, 210, 214, 218, 222, 226 and 230, and/or a light chain amino acid sequence comprising at least one of the CDR's selected from the group consisting of: (d) CDRL1's as shown in SEQ ID NOS:4, 8, 12, 16, 20, 24, 28, 32, 38, 44, 48, 52, 56, 58, 64, 68, 72, 76, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228 and 232, (e) CDRL2's as shown in SEQ ID NOS:4, 8, 12, 16, 20, 24, 28, 32, 38, 44, 48, 52, 56, 58, 64, 68, 72, 76, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228 and 232, and (f) CDRL3's as shown in SEQ ID NOS:4, 8, 12, 16, 20, 24, 28, 32, 38, 44, 48, 52, 56, 58, 64, 68, 72, 76, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228 and 232, as shown in the sequence listing filed herewith. An antigen-binding protein for use according to the invention is as defined in the claims. Other antigen-binding proteins are provided as comparative examples.

[0033] An isolated binding protein targeted to HER-3 can include a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOS:2, 6, 10, 14, 18, 22, 26, 30, 34, 36, 40, 42, 46, 50, 54, 60, 62, 66, 70, 74, 78, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 122, 126, 130, 134, 138, 142, 146, 150, 154, 158, 162, 166, 170, 174, 178, 182, 186, 190, 194, 198, 202, 206, 210, 214, 218, 222, 226 and 230, and/or a light chain amino acid sequence selected from the group consisting of SEQ ID NOS:4, 8, 12, 16, 20, 24, 28, 32, 38, 44, 48, 52, 56, 58, 64, 68, 72, 76, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228 and 232, as shown in the sequence listing filed herewith.

[0034] An anti-HER-3 antibody can include a heavy chain amino acid sequence and a light chain amino acid sequence as shown in SEQ ID NOS:2 and 4, 6 and 8, 10 and 12, 14 and 16, 18 and 20, 22 and 24, 26 and 28, 30 and 32, 36 and 38, 42 and 44, 46 and 48, 50 and 52, 54 and 56, 60 and 58, 62 and 64, 66 and 68, 70 and 72, 74 and 76, 78 and 82, 80 and 82, 84 and 86, 88 and 90, 92 and 94, 96 and 98, 100 and 102, 104 and 106, 108 and 110, 112 and 114, 116 and 118, 122 and 124, 126 and 128, 130 and 132, 134 and 136, 138 and 140, 142 and 144, 146 and 148, 150 and 152, 154 and 156, 158 and 160, 162 and 164, 166 and 168, 170 and 172, 174 and 176, 178 and 180, 182 and 184, 186 and 188, 190 and 192, 194 and 196, 198 and 200, 202 and 204, 206 and 208, 210 and 212, 214 and 216, 218 and 220, 222 and 224, 226 and 228, 230 and 232, or a heavy chain amino acid sequence as shown in any one of SEQ ID NOS:34, 40, 60, 62, and 120, or a light chain amino acid sequence as shown in either of SEQ ID NOS: 58 and 64, as shown in the sequence listing filed herewith.

[0035] A protein targeted to HER-3 can be a scaffold protein having an antibody-like binding activity (e.g., having activity similar to an anti-HER-3 antibody), or an antibody, *e.g.,* an anti-HER-3 antibody. Exemplary anti-HER-3 antibodies are designated U1-1, U1-2, U1-3, U1-4, U1-5, U1-6, U1-7, U1-8, U1-9, U1-10, U1-11, U1-12, U1-13, U1-14, U1-15, U1-16, U1-17, U1-18, U1-19, U1-20, U1-21, U1-22, U1-23, U1-24, U1-25, U1-26, U1-27, U1-28, U1-29, U1-30, U1-31, U1-32, U1-33, U1-34, U1-35, U1-36, U1-37, U1-38, U1-39, U1-40, U1-41, U1-42, U1-43, U1-44, U1-45, U1-46, U1-47, U1-48, U1-49, U1-50, U1-51, U1-52, U1-53, U1-55.1, U1-55, U1-57.1, U1-57, U1-58, U1-59, U1-61.1, U1-61, and U1-62, or an antibody having at least one heavy or light chain of one of the aforesaid antibodies. The antibody designated U1-59 (SEQ ID NO: 70/72)can beused according to the present invention.

[0036] It is to be understood that the amino acid sequence of the HER-3 binding proteins described herein is not limited to the twenty conventional amino acids (*see,* Immunology - A Synthesis (2nd Edition, Golub and Gren, eds., Sinauer Associates, Sunderland, Mass. (1991). For example, the amino acids may include stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α-,α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids. Examples of unconventional amino acids, which may also be suitable components for the binding proteins provided herein, include: 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and imino acids, *e.g.*, 4-hydroxyproline.

[0037] Furthermore, minor variations in the amino acid sequences shown in SEQ ID NOS:70 and 72 (as set forth in the appendix filed herewith) are contemplated as being encompassed by the present disclosure, provided that the variations are not in the CDR sequences defined in the claims and maintain at least 75% (*e.g.,* at least 80%, 90%, 95%, or 99%) of the sequences shown in SEQ ID NOS:70 and 72. Variations can occur within the framework regions (*i.e.*, outside the CDRs). In some embodiments, variations in the amino acid sequences, *i.e.,* deletions, insertions and/or substitutions of at least one amino acid, can occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other binding proteins of known structure and/or function. Methods for identifying protein sequences that fold into a known three-dimensional structure are known in the art. (*See, e.g.,* Bowie et al. (1991) Science 253:164; Proteins, Structures and Molecular Principles, Creighton, Ed., W H. Freeman and Company, New York (1984); Introduction to Protein Structure, Branden and Tooze, eds., Garland Publishing, New York, N.Y. (1991);

and Thornton et al. (1991) Nature 354:105) Thus, those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the proteins described herein.

[0038] Variations in the amino acid sequences shown in SEQ ID NOS:70 and 72 can include those that lead to a reduced susceptibility to proteolysis or oxidation, alter glycosylation patterns or alter binding affinities or confer or modify other physicochemical or functional properties of the binding protein. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Amino acid families include the following: acidic family = aspartate, glutamate; basic family = lysine, arginine, histidine; non-polar family = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and uncharged polar family = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Alternative families include: aliphatic-hydroxy family = serine and threonine; amide-containing family = asparagine and glutamine; aliphatic family = alanine, valine, leucine and isoleucine; and aromatic family = phenylalanine, tryptophan, and tyrosine. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting binding protein, especially if the replacement does not involve an amino acid within a framework site. However, all other possible amino acid replacements also are encompassed herein. Whether an amino acid change results in a functional HER-3 binding protein that reduces signal transduction of HER-3 can readily be determined by assaying the specific HER-3 binding activity of the resulting binding protein by ELISA or FACS, or *in vitro* or *in vivo* functional assays.

[0039] In some embodiments, a HER-3 binding protein can be coupled to an effector group. Such a binding protein can be especially useful for therapeutic applications. As used herein, the term "effector group" refers to a cytotoxic group such as a radioisotope or radionuclide, a toxin, a therapeutic group or other effector group known in the art. Examples of suitable effector groups are radioisotopes or radionuclides (*e.g.*, ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I) or non-radio isotopes (*e.g.*, 2D), calicheamicin, dolastatin analogs such as auristatins, and chemotherapeutic agents such as geldanamycin and maytansine derivates, including DM1. Thus, in some cases, a group can be both a labeling group and an effector group. Various methods of attaching effector groups to polypeptides or glycopolypeptides (such as antibodies) are known in the art, and may be used in making and carrying out the compositions and methods described herein. In some embodiments, it may be useful to have effector groups attached to a binding protein by spacer arms of various lengths to, for example, reduce potential steric hindrance.

[0040] This document also relates to processes for preparing an isolated HER-3 binding protein, comprising the step of preparing the protein from a host cell that expresses the protein. Host cells that can be used include, without limitation, hybridomas, eukaryotic cells (*e.g.*, mammalian cells such as hamster, rabbit, rat, pig, or mouse cells), plant cells, fungal cells, yeast cells (*e.g., Saccharomyces cerevisiae* or *Pichia pastoris* cells), prokaryotic cells (*e.g., E. coli* cells), and other cells used for production of binding proteins. Various methods for preparing and isolating binding proteins, such as scaffold proteins or antibodies, from host cells are known in the art and may be used in performing the methods described herein. Moreover, methods for preparing binding protein fragments, *e.g.*, scaffold protein fragments or antibody fragments, such as papain or pepsin digestion, modern cloning techniques, techniques for preparing single chain antibody molecules (Plückthun, *supra*) and diabodies (Hollinger *et al., supra*), also are known to those skilled in the art and may be used in performing the presently described methods.

[0041] In some embodiments, a HER-3 binding protein can be prepared from a hybridoma that secretes the protein. *See, e.g.,* Köhler et al. (1975) Nature 256:495.

[0042] In some embodiments, a HER-3 binding protein can be prepared recombinantly by optimizing and/or amplifying expression of the binding protein in host cells, and isolating the binding protein from the host cells. To this end, host cells can be transformed or transfected with DNA (*e.g.,* a vector) encoding a HER-3 binding protein, and cultured under conditions appropriate to produce the binding protein. *See, e.g.,* US Patent No. 4,816,567. Useful host cells include, for example, CHO cells, NS/0 myeloma cells, human embryonic kidney 293 cells, *E. coli* cells, and *Saccharomyces cerevisiae* cells.

[0043] HER-3 binding proteins that are antibodies can be prepared from animals genetically engineered to make fully human antibodies, or from an antibody display library made in bacteriophage, yeast, ribosome or *E. coli. See, e.g.,* Clackson et al. (1991) Nature 352:624-628; Marks et al. (1991) J. Mol. Biol. 222:581-597; Feldhaus and Siegel (2004) J. Immunol. Methods 290:69-80; Groves and Osbourn (2005) Expert Opin. Biol. Ther. 5:125-135; and Jostock and Dubel (2005) Comb. Chem. High Throughput Screen 8:127-133.

[0044] In some embodiments, antibodies as provided herein can be fully human or humanized antibodies. Human antibodies avoid certain problems associated with xenogeneic antibodies, such as antibodies that possess murine or rat variable and/or constant regions. The presence of xenogeneic-derived proteins can lead to an immune response against the antibody by a patient, subsequently leading to the rapid clearance of the antibody, loss of therapeutic utility through neutralization of the antibody, and/or severe, even life-threatening, allergic reactions. To avoid the using murine or rat-derived antibodies, fully human antibodies can be generated through the introduction of functional human antibody

loci into a rodent or another mammal or animal so that the rodent, other mammal or animal produces fully human antibodies.

[0045] One method for generating fully human antibodies is to utilize XENOMOUSE® strains of mice that have been engineered to contain 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus. Other XENOMOUSE® strains of mice contain 980 kb and 800 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus. Still other XENOMOUSE® strains of mice contain 980 kb and 800 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus plus a 740 kb-sized germline configured complete human lambda light chain locus. *See,* Mendez et al. (1997) Nature Genetics 15:146-156; and Green and Jakobovits (1998) J. Exp. Med. 188:483-495. XENOMOUSE® strains are available from Amgen, Thousand Oaks, CA.

[0046] The production of XENOMOUSE® mice is further discussed and delineated in US Patent Publication 2003/0217373, filed November 20, 2002; US Patent Nos. 5,939,598, 6,075,181, 6,114,598, 6,150,584, 6,162,963, 6,673,986, 6,833,268, and 7,435,871, and Japanese Patent Nos. 3068180B2, 3068506B2, and 3068507B2. *See,* also, European Patent No. EP0463151, PCT Publication Nos. WO 94/02602, WO 96/34096, WO 98/24893, and WO 00/76310.

[0047] Alternatively, a "minilocus" approach can be used. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and a second constant region (*e.g.,* a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in US Patent Nos. 5,545,806, 5,545,807, 5,569,825, 5,591,669, 5,612,205, 5,625,126, 5,625,825, 5,633,425, 5,643,763, 5,661,016, 5,721,367, 5,770,429, 5,789,215, 5,789,650, 5,814,318, 5,874,299, 5,877,397, 5,981,175, 6,023,010, 6,255,458. *See,* also, EP Patent No. 0546073, and PCT Publication Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884.

[0048] Human antibodies also can be generated from mice in which, through microcell fusion, large pieces of chromosomes, or entire chromosomes, have been introduced. *See,* EP Patent Application Nos. 773288 and 843961, the disclosures of which are hereby incorporated herein by reference in their entireties. Additionally, KM™ mice, which are the result of cross-breeding of Kirin's Tc mice with Medarex's minilocus (Humab) mice have been generated. These mice possess the HC transchromosome of the Kirin mice and the kappa chain transgene of the Medarex mice (Ishida *et al.* (2002) *Cloning Stem Cells* 4:91-102).

[0049] Human antibodies also can be derived by *in vitro* methods. Suitable examples include, but are not limited to, phage display (as commercialized by Cambridge Antibody Technology, Morphosys, Dyax, Biosite/Medarex, Xoma, Symphogen, Alexion (formerly Proliferon), and Affimed), ribosome display (as commercialized by Cambridge Antibody Technology), yeast display, and the like.

[0050] As described herein, antibodies were prepared using XENOMOUSE® technology, as described below. Such mice are capable of producing human immunoglobulin molecules and antibodies, and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed herein. For example, transgenic production of mice and antibodies therefrom is disclosed in US Patent Application Serial No. 08/759,620, filed December 3, 1996, and PCT Publication Nos. WO 98/24893 and WO 00/76310. *See also* Mendez et al. (1997) Nature Genetics 15:146-156.

[0051] Using technology as described herein, fully human monoclonal antibodies to a variety of antigens can be produced. For example, XENOMOUSE® lines of mice can be immunized with a HER-3 antigen of interest (*e.g.*, HER-3 or a fragment thereof), lymphatic cells (such as B-cells) can be recovered from mice that express antibodies, and the recovered cell lines can be fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. These hybridoma cell lines can be screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. Provided herein are methods for the production of multiple hybridoma cell lines that produce antibodies specific to HER-3. Further provided herein are methods for characterizing antibodies produced by such cell lines, including nucleotide and amino acid sequence analyses of the heavy and light chains of such antibodies.

[0052] In general, antibodies produced by fused hybridomas as described below are human IgG1 heavy chains with fully human kappa light chains, although some antibodies described herein possess human IgG4 heavy chains as well as IgG1 heavy chains. Antibodies also can be of other human isotypes, including IgG2 and IgG3. The antibodies generally have high affinities, with a K_D typically from about 10^{-6} to about 10^{-13} M or below, when measured by solid phase and cell-based techniques.

[0053] This document also describes isolated nucleic acid molecules that encode HER-3 binding proteins as described herein. The term "isolated nucleic acid molecule," as used herein, refers to a polynucleotide of genomic, cDNA, or synthetic origin, or some combination thereof, which (1) is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide to which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence. Further, the term "nucleic acid molecule," as used herein, means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide, such as nucleotides with modified or substituted sugar groups

and the like. The term also includes single and double stranded forms of DNA.

[0054] A nucleic acid molecule can be operably linked to a control sequence. The term "control sequence," as used herein, refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include promoters, ribosomal binding sites, and transcription termination sequences. In eukaryotes, generally, such control sequences include promoters and transcription termination sequences. The term "control sequence" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Furthermore, the term "operably linked", as used herein, refers to positions of components so described which are in a relationship permitting them to function in their intended manner. Moreover, an expression control sequence operably linked to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the expression control sequence.

[0055] Also described herein are vectors comprising a nucleic acid molecule encoding a binding protein as described herein. The nucleic acid molecule can be operably linked to a control sequence. Furthermore, the vector may additionally contain a replication origin or a selection marker gene. Examples of vectors that may be used include, *e.g.*, plasmids, cosmids, phages, and viruses.

[0056] This document also describes host cells transformed with a nucleic acid molecule or vector as described herein. Transformation can be accomplished by any known method for introducing polynucleotides into a host cell, including, for example, packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector), or by transfection procedures known in the art, as exemplified by US Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art, and include, without limitation, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. Examples of host cells that may be used include hybridomas, eukaryotic cells (*e.g.*, mammalian cells such as hamster, rabbit, rat, pig, mouse, or other animal cells), plant cells (*e.g.*, corn and tobacco cells), fungal cells (*e.g., S. cerevisiae* and *P. pastoris* cells), prokaryotic cells such as *E. coli,* and other cells used in the art for production of antibodies. Mammalian cell lines available as hosts for expression are well known in the art and include, for example, many immortalized cell lines available from the American Type Culture Collection (ATCC; Manassas, VA). These include, without limitation, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.,* Hep G2 cells), and a number of other cell lines.

3. Agents that bind to other HER family members

[0057] As outlined above, the compositions and methods described herein for treatment of HER-3 associated disease include a first agent that binds to HER-3, in combination with a second agent that binds and/or inhibits at least one other member of the HER family, including but not limited to, EGF-R, HER-2, HER-4. According to the invention, the second agent is trastuzumab. Other examples outside the scope of the invention include biological drug, *e.g.,* a binding protein, such as an antibody specifically binding to a member of the HER family, a small molecular compound that binds to and/or alters (*e.g.,* inhibits) the activity of at least one member of the HER family other than (or in addition to) HER-3, an siRNA, or a natural substance. As used herein, the terms "other HER family members" and "another HER family member" refer to HER family members that are not HER-3. Examples are the EGF-R, HER-2, and HER-4, but "HER family member" also includes family members that have not yet been identified.

[0058] The second agent can alter the activity (*e.g.*, increase or decrease) the activity of the other HER family member, either through a direct effect or an indirect effect on the HER family member. It is noted, however, that all second agents as described herein will have an effect on HER family function and activity.

[0059] Trastuzumab (also known as HERCEPTIN®) is a humanized monoclonal antibody that interferes with the HER2/neu receptor. According to the invention, a composition for treatment of HER3-associated disease which is HER3-expressing or overexpressing cancer can be U1-59 in combination with trastuzumab, or U1-59, in combination with trastuzumab and other agent(s) such as docetaxel or paclitaxel, for treatment of HER3-expressing or overexpressing cancer, such as breast cancer, gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, renal cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma, metastatic breast cancer, non-small cell lung cancer, epidermoid carcinoma, fibrosarcoma, melanoma, nasopharyngeal carcinoma, or squamous cell carcinoma. In some preferred embodiments, U1-59 can be used in the treatment of patients with cancers including breast cancer and metastatic breast cancer whose tumors express or overexpress the HER-2 protein and who have not received chemotherapy for their (metastatic) disease, in combination with trastuzumab and paclitaxel, or, in combination with trastuzumab and docetaxel.

4. Additional Agents to Be Used in the Compositions and Methods Disclosed Herein

[0060] Additional agents may be added to the first and second agent as described herein. These, in some embodiments, will be chemotherapeutic drugs.

[0061] For example, agents that act as microtubule stimulants include NK-105(paclitaxel) [(-)-(1S,2R,3S,4S,SR,7S,8S,10R,13S)-4,10-diacetoxy-2-benzoyloxy-5,20-epoxy-1,7-dihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-benzoylamino-2-hydroxy-3-phenylpropionate] (NanoCarrier, Chiba, Japan), milataxel (1,10β-dihydroxy-9-oxo-5β,20-epoxy-3zeta-tax-11-ene-2α,4,7β13α-tetrayl 4-acetate 2-benzoate 13-[(2R,3R)-3-(tert-butoxycarbonylamino)-3-(furan-2-yl)-2-hydroxypropanoate] 7-propanoate) (Taxolog, Fairfield, NJ), laulimalide (Kosan Biosciences, Hayward, CA (B-M Squibb)), sarcodictyin A (3-(1-methylimidazol-4-yl)-2(E)-propenoic acid (1R,4aR,6S,7S,10R,12aR)-11-methoxycarbonyl-7,10-epoxy-10-hydroxy-1-isopropyl-4,7-dimethyl-1,2,4a,5,6,7,10,12a-octahydrobenzocyclododecen-6-yl ester) (Pfizer, New York, NY), simotaxel ((2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxete-6,9,12,12b-tetrayl 12b-acetate 12-benzoate 6-cyclopentanecarboxylate 9-[(2R,3R)-2-hydroxy-3-[[(1-methylethoxy)carbonyl]amino]-3-(thiophen-2-yl)propanoate]) (Taxolog, Fairfield, NJ), SYN-2001 (CLL Pharma, Nice, France), TL-310 (Taxolog, Fairfield, NJ), TL1836 (Taxolog, Fairfield, NJ), tesetaxel (2'-[(dimethylamino)methyl]-1-hydroxy-5β,20-epoxy-9α,10α-dihydro[1,3]dioxolo[4',5':9,10]tax-11-ene-2α,4,13α-triyl4-acetate 2-benzoate 13-[(2R,3S)-3-[(tert-butoxycarbonyl)amino]-3-(3-fluoropyridin-2-yl)-2-hydroxypropanoate) (Daiichi Sankyo, Tokyo, Japan), TL-1892 (Taxolog, Fairfield, NJ), TPI-287((2'R,3'S)-2'-hydroxy-N-carboxy-3'-amino-5'-methyl-hexanoic, N-tert-butyl ester, 13 ester 5β-20-epoxy-1,2α,4,7β,9α,10α,13α-heptahydroxy-4,10-diacetate-2-benzoate-7,9-acrolein acetal-tax-11-ene (Tapestry Pharmaceuticals, Boulder, CO), ortataxel (2aR-[2aα,4β,4aβ,6β,9α(2R,3S),10β,11β, 12α,12aα,12bα]-3-(tert-butoxycarbonylamino)-2-hydroxy-5-methyl-hexanoic acid 6,12b-diacetoxy-12-benzoyloxy-10,11-carbonyldioxy-4-hydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benz[1,2-b]oxet-9-yl ester) (Indena, Milan, Italy), paclitaxel poliglumex (L-pyroglutamylpoly-L-glutamyl-L-glutamic acid partially γ-esterified with (1R,2S)-2-(benzoylamino)-1-[[[(2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-6,12b-bis(acetyloxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-7,11-methano-1H-cyclodeca[3,4]benzo[1,2-b]oxet-9-yl]oxy]carbonyl]-2-phenylethyl)(Cell Therapeutics, Seattle, WA), paclitaxel protein-bound particles (paclitaxel : (-)-(1S,2R,3S,4S, 5R,7S,8S,10R,13S)-4,10-diacetoxy-2-benzoyloxy-5,20-epoxy-1,7-dihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-benzoylamino-2-hydroxy-3-phenylpropionate) (Abraxis BioScience, Los Angeles, CA), paclitaxel(NCI)((-)-(1S,2R,3S,4S,5R,7S,8S,10R,13S)-4,10-diacetoxy-2-benzoyloxy-5,20-epoxy-1,7-dihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-benzoylamino-2-hydroxy-3-phenylpropionate) (NCI(NIH)), paclitaxel (NeoPharm, Lake Bluff, IL)((-)-(1S,2R,3S,4S,5R,7S,8S,10R,13S)-4,10-diacetoxy-2-benzoyloxy-5,20-epoxy-1,7-dihydroxy-9-oxotax-11-en-13-yl (2R,3S)-3-benzoylamino-2-hydroxy-3-phenylpropionate) (NeoPharm, Lake Bluff, IL), patupilone((1S,3S,7S,10R,11S,12S,16R)-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[(1E)-1-(2-methyl-1,3-thiazol-4-yl)prop-1-en-2-yl]-4,17-dioxabicyclo[14.1.0] heptadecane-5,9-dione) (US Publication No. 2003/0104625, Novartis, Basel, Switzerland), PEG-paclitaxel (Enzo Pharmaceuticals, Long Island, NY), docetaxel hydrate((-)-(1S,2S,3R, 4S,5R,7S,8S,10R,13S)-4-acetoxy-2-benzoyloxy-5,20-epoxy-1,7,10-trihydroxy-9-oxotax-11-ene-13-yl(2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate trihydrate) (Sanofi-Aventis, Bridgewater, NJ), eleutherobin (3-(1-methylimidazol-4-yl)-2(E)-propenoic acid (1R,4aR,6S,7S,10R,12aR)-11-(2-O-acetyl-β-D-arabinopyranosyloxymethyl)-7,10-epoxy-1-isopropyl-10-methoxy-4,7-dimethyl-1,2,4a,5,6,7,10,12a-octahydrobenzocyclo-dodecen-6-yl ester) (Bristol-Myers Squibb, New York, NY), IDN-5390 (Indena, Milan, Italy), ixabepilone ((1S,3S,7S,10R,11S,12S,16R)-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[(1E)-1-methyl-2-(2-methylthiazol-4-yl)ethenyl]-17-oxa-4-azabicyclo[14.1.0]heptadecane-5,9-dione) (Bristol-Myers Squibb, New York, NY), KOS-1584 (Kosan Biosciences, Hayward, CA(B-M Squibb)), KOS-1803 (17-iso-oxazole 26-trifluoro-9,10-dehydro-12,13-desoxy-epothilone B) (Kosan Biosciences, Hayward, CA (B-M Squibb)), KOS-862 (Kosan Biosciences, Hayward, CA (B-M Squibb); US Patent Nos. 6204388 and 6303342), larotaxel (1-hydroxy-9-oxo-5β,20-epoxy-7β,19-cyclotax-11-ene-2α,4,10β,13α-tetrayl 4,10-diacetate 2-benzoate 13-[(2R,3S)-3-[(tert-butoxycarbonyl)amino]-2-hydroxy-3-phenylpropanoate] dehydrate) (Sanofi-Aventis, Bridgewater, NJ, PCT Publication Nos. WO 95/26961 and WO 96/1259), ANG-1005 (Angiopep-2/paclitaxel conjugate) (AngioChem, Montreal, Canada, US Patent No. 7557182), BMS-184476 (Bristol-Myers Squibb, New York, NY, EP Publication No. 639577), BMS-188797 (Bristol-Myers Squibb, New York, NY), BMS-275183 (3'-tert-butyl-3'-N-tert-butyloxycarbonyl-4-deacetyl-3'-dephenyl-3'-N-debenzoyl-4-O-methyoxy-carbonyl-paclitaxel) (Bristol-Myers Squibb, New York, NY), BMS-310705 (Bristol-Myers Squibb, New York, NY), BMS-753493 (Bristol-Myers Squibb, New York, NY), cabazitaxel (1-hydroxy-7β,10β-dimethoxy-9-oxo-5β,20-epoxytax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-[(2R,3S)-3-[[(tertbutoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate]) (Sanofi-Aventis, Bridgewater, NJ), DHA-paclitaxel (Protarga, King of Prussia, PA, TAXOPREXIN®), disermolide ([3S-[3α,4β,5β,6α(2R*,3Z,5R*,6R*,7S*,8Z,11R*,12S*, 13S*,14S*,15R*,16E)]]-6-[14[(aminocarbonyl)oxy]-2,6,12-trihydroxy-5,7,9,11,13,15-hexamethyl-3,8,16,18-nonadecatetraenyl]tetrahydro-4-hydroxy-3,5-dimethyl-2H-pyran-2-one) (Novartis, Basel, Switzerland, US Patent Nos. 4939168 and 5681847). Some of these microtubule stimulants have a

taxane ring in their chemical structures; such compounds having a taxane ring are referred as "taxanes" herein.

[0062] Anthracyclins include actinomycins such as actinomycin D (Dactinomycin: 2-amino-N,N'-bis[(6S,9R,10S,13R,18aS)- 6,13-diisopropyl- 2,5,9-trimethyl- 1,4,7,11,14-pentaoxohexadecahydro- 1H-pyrrolo[2,1-i][1,4,7,10,13] oxatetraazacyclohexadecin- 10-yl]-4,6-dimethyl- 3-oxo- 3H-phenoxazine- 1,9-dicarboxamide), bleomycin (bleomycin hydrochloride: (3-{[(2'-{(5S,8S,9S,10R,13S)-15-{6-amino-2- [(1S)-3-amino-1-{[(2S)-2,3-diamino-3-oxopropyl]amino}-3-oxopropyl] -5-methylpyrimidin-4-yl}-13-[{[(2R,3S,4S,5S,6S)-3- {[(2R,3S,4S,5R,6R)-4-(carbamoyloxy)-3,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl]oxy} -4,5-dihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl]oxy} (1H-imidazol-5-yl)methyl]-9-hydroxy-5-[(1R)-1-hydroxyethyl]-8,10-dimethyl-4,7,12,15- tetraoxo-3,6,11,14-tetraazapentadec-1-yl}-2,4'-bi-1,3- thiazol-4-yl)carbonyl]amino}propyl)(dimethyl)sulfonium), daunorubicin hydrochloride (daunorubicin: 8S-cis)-8-Acetyl-10-((3-amino-2,3,6-trideoxy-alpha-L-lyxo-hexopyranosyl) oxy)-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride), doxorubicin hydrochloride (doxorubicin: (8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6, 8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride) (Alza, Mountain View, CA), idarubicin hydrochloride ((7S,9S)-9-acetyl-7,8,9,10-tetrahydro-6,7,9,11-tetrahydroxy-7-O-(2,3,6-trideoxy-3-amino-α-L-lyxo-hexopyranosyl)-5,12-naphthacenedione hydrochloride) (Pfizer, New York, NY, US Patent Nos. 4046878 and 4471052), and mitomycin ((1aS,8S,8aR,8bR)-6-Amino-4,7-dioxo-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methylazirino[2,3:3,4]pyrrolo[1,2-α]indol-8-ylmethylcarbamate) (Kyowa-Hakko-Kirin, Tokyo, Japan).

[0063] Cisplatin and gemcitabine are chemotherapeutic agents. Cisplatin or cis-diamminedichloroplatinum(II) is a platinum-based drug used to treat various types of cancers. The cisplatin platinum complex reacts *in vivo*, binding to and causing crosslinking of DNA, which ultimately triggers apoptosis. Gemcitabine is a nucleoside analog in which the hydrogen atoms on the 2' carbons of deoxycytidine are replaced by fluorine atoms. Like fluorouracil and other pyrimidine analogues, gemcitabine replaces cytidine during DNA replication, which arrests tumor growth since further nucleosides cannot be attached to the "faulty" nucleoside, resulting in apoptosis. Gemcitabine is marketed as GEMZAR® by Eli Lilly and Company (Indianapolis, IN). In some embodiments, a combination for treatment of HER3-associated disease can be: U1-59 in combination with trastuzumab as described herein and cisplatin or gemcitabine and other agent(s), for treatment of cancer which is gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, kidney cancer, colon cancer, thyroid cancer, bladder cancer, glioma, melanoma, lung cancer including non-small cell lung cancer, colorectal cancer and/or breast cancer including metastatic breast cancer.

[0064] Capecitabine (pentyl[1-(3,4-dihydroxy-5-methyl-tetrahydrofuran-2-yl)-5-fluoro-2-oxo-1H-pyrimidin-4-yl]aminomethanoate, Xeloda, Roche) is an orally-administered chemotherapeutic agent. Capecitabine is a prodrug that is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue. In some embodiments, a combination for treatment of HER3-associated disease can be: U1-59 in combination with trastuzumab and capecitabine for treatment of cancer, wherein the cancer is gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, kidney cancer, colon cancer, thyroid cancer, bladder cancer, glioma, melanoma, lung cancer including non-small cell lung cancer, colorectal cancer and/or breast cancer including metastatic breast cancer. In some cases, such a combination can be administered after failure of prior treatment with an anthracyclin or taxane, for example. In some preferred embodiments, U1-59 can be used in the treatment of patients with cancers including breast cancer and metastatic breast cancer whose tumors express or overexpress the HER-2 protein and who have received prior chemotherapy including an anthracycline (for example, doxorubicin or related agent), and/or a taxane (for example, paclitaxel or docetaxel), and trastuzumab, in combination with lapatinib and capecitabine.

[0065] Docetaxel((2*R*,3*S*)-*N*-carboxy-3-phenylisoserine, *N*-tert-butyl ester, 13-ester with 5, 20-epoxy-1, 2, 4, 7, 10, 13-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate) and paclitaxel((2α,4α,5β,7β,10β,13α)-4,10-bis(acetyloxy)-13-{[(2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]oxy}-1,7-dihydroxy-9-oxo-5,20-epoxytax-11-en-2-yl be) are chemotherapeutic agents. Docetaxel is marketed as Taxotere by Sanofi Aventis. Paclitaxel is marketed as Taxol by Bristol-Myers Squibb. In the formulation of Taxol, paclitaxel is dissolved in Cremophor EL and ethanol, as a delivery agent. A formulation in which paclitaxel is bound to albumin is marketed as Abraxane. In some embodiments, a combination for treatment of HER3-associated disease can be: U1-59 in combination with trastuzumab and docetaxel or paclitaxel and other agent(s) for treatment of cancer, wherein the cancer is gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, kidney cancer, colon cancer, thyroid cancer, bladder cancer, glioma, melanoma, lung cancer including non-small cell lung cancer, colorectal cancer and/or breast cancer including metastatic breast cancer. In some preferred embodiments U1-59 can be used in the treatment of patients with cancers including breast cancer and metastatic breast cancer whose tumors express or overexpress the HER-2 protein and who have not received chemotherapy for their (metastatic) disease, in combination with trastuzumab and paclitaxel, or in combination with trastuzumab and docetaxel.

[0066] Doxorubicin hydrochloride liposome injection is marketed as Doxil, a liposome formulation comprising doxorubicin chloride. In some embodiments, a combination treatment for HER-3-associated disease can include administering

U1-59 in combination with trastuzumab and doxorubicin hydrochloride liposome injection, with or without one or more other agents such as paclitaxel or platinum-based chemotherapeutic agents, for treatment of cancer such as breast cancer, gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, renal cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma, metastatic breast cancer, non-small cell lung cancer, epidermoid carcinoma, fibrosarcoma, melanoma, nasopharyngeal carcinoma, and squamous cell carcinoma.

[0067] Irinotecan hydrochloride hydrate (irinotecan: (+)-(4S)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino)carbonyloxy]-1H-pyrano[3',4':6,7]indolizino[1-2-b]quinoline-3,14(4H,12H)-dione hydrochloride trihydrate) (Yakult, EP Publication Nos. 137145 and 56692) is marketed as Campto, Camptosar and Ircan. In some embodiments, a combination treatment for HER3-associated disease can include administering U1-59 in combination with trastuzumab and irinotecan hydrochloride hydrate, or U1-59 in combination with trastuzumab, irinotecan hydrochloride hydrate, and one or more other agent(s) such as 5-FU(5'-deoxy-5-fluorouridine or 5-fluoro-2,4(1H,3H)-pyrimidinedione), calcium folinate (N-[4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methylamino]benzoyl]-L-glutamic acid calcium salt (1:1)) or calcium levofolinate ((-)-calcium N-[4-[[[(6S)-2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl]methyl]amino]benzoyl]-L-glutamate), and combinations thereof, for treatment of cancer such as breast cancer, gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, renal cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma, metastatic breast cancer, non-small cell lung cancer, epidermoid carcinoma, fibrosarcoma, melanoma, nasopharyngeal carcinoma, and squamous cell carcinoma.

[0068] In some embodiments, the additional agents to be use in the compositions and methods described herein may be an artificial or naturally-occurring scaffold which is not an antibody, but has an antibody-like activity (e.g., has an activity similar to that of an antibody).

[0069] In some other embodiments, said additional agents can be agents inhibit, block or reduce (act as antagonists towards), or, activate, stimulate or accelerate (act as agonist towards) an activity of other targets, including but not limited to those affect cellular growth and/or survival pathways, such as PI3K inhibitors, AKT inhibitors, mTOR inhibitors, RAF/B-RAF inhibitors, RAS inhibitors, MEK inhibitors, Death Receptor inhibitors including DR4 and DR5 agonists such as anti-DR4 or DR5 agonistic antibodies (for example, cedelizumab, tigatuzumab, drozirumab, conatumumab), PPAR gamma agonists (for example, efatutazone, troglitazone, pioglitazone, rosiglitazone), c-MET inhibitors, Hsp-90 inhibitors and telomerase inhibitors.

[0070] Said additional agents can be anti-angiogenics, including but not limited to, VEGF antagonists/inhibitors (for example, bevacizumab, vandetanib).

[0071] Said additional agents can be immunotherapeutic such as vaccines or cellular therapeutics.

[0072] As further described below, these and other agents can be contained within the compositions described herein, and can be administered in a variety of different forms, combinations and dosages.

5. Compositions

[0073] This document also describes pharmaceutical compositions comprising a HER-3 binding agent as described herein, in combination with a second agent that is directed against another HER family protein or is a chemotherapeutic compound, as well as one or more pharmaceutically acceptable carriers, diluents and/or adjuvants. The term "pharmaceutical composition," as used herein, refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient (The McGraw-Hill Dictionary of Chemical Terms, Parker, Ed., McGraw-Hill, San Francisco (1985)). The potency of the pharmaceutical compositions provided herein typically is based on the binding of the at least one binding protein to HER-3. This binding can lead to a reduction of the HER-3-mediated signal transduction.

[0074] A "pharmaceutically acceptable carrier" (also referred to herein as an "excipient" or a "carrier") is a pharmaceutically acceptable solvent, suspending agent, stabilizing agent, or any other pharmacologically inert vehicle for delivering one or more therapeutic compounds (*e.g.*, HER binding proteins) to a subject, which is nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Pharmaceutically acceptable carriers can be liquid or solid, and can be selected with the planned manner of administration in mind so as to provide for the desired bulk, consistency, and other pertinent transport and chemical properties, when combined with one or more of therapeutic compounds and any other components of a given pharmaceutical composition. Typical pharmaceutically acceptable carriers that do not deleteriously react with amino acids include, by way of example and not limitation: water, saline solution, binding agents (*e.g.*, polyvinylpyrrolidone or hydroxypropyl methylcellulose), fillers (*e.g.*, lactose and other sugars, gelatin, or calcium sulfate), lubricants (e.g., starch, polyethylene glycol, or sodium acetate), disintegrates (e.g., starch or sodium starch glycolate), and wetting agents (e.g., sodium lauryl sulfate). Pharmaceutically acceptable carriers also include aqueous pH buffered solutions or liposomes (small vesicles composed of various types of lipids, phospholipids and/or surfactants which are useful for delivery of a drug to a mammal). Further examples of pharmaceutically

acceptable carriers include buffers such as phosphate, citrate, and other organic acids, antioxidants such as ascorbic acid, 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, arginine or lysine, monosaccharides, disaccharides, and other carbohydrates including glucose, mannose or dextrins, chelating agents such as EDTA, sugar alcohols such as mannitol or sorbitol, salt-forming counterions such as sodium, and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0075] Liposomes are vesicles that have a membrane formed from a lipophilic material and an aqueous interior that can contain the composition to be delivered. Liposomes can be particularly useful due to their specificity and the duration of action they offer from the standpoint of drug delivery. Liposome compositions can be formed, for example, from phosphatidylcholine, dimyristoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol, or dioleoyl phosphatidylethanolamine. Numerous lipophilic agents are commercially available, including LIPOFECTIN® (Invitrogen/Life Technologies, Carlsbad, CA) and EFFECTENE™ (Qiagen, Valencia, CA).

[0076] At least one of the agents contained in a pharmaceutical composition (*e.g.*, a HER-3 binding agent or an agent that binds and/or inhibits another HER family member) can be coupled to an effector such as calicheamicin, duocarmycins, auristatins, maytansinoids, a radioisotope, or a toxic chemotherapeutic agent such as geldanamycin and maytansine. Such conjugates can be particularly useful for targeting cells (*e.g.,* cancer cells) expressing HER-3.

[0077] Linking binding proteins to radioisotopes can provide advantages to tumor treatments. Unlike chemotherapy and other forms of cancer treatment, radioimmunotherapy or the administration of a radioisotope-binding protein combination can directly target cancer cells with minimal damage to surrounding normal, healthy tissue. With this "magic bullet," patients can be treated with much smaller quantities of radioisotopes than other forms of treatment available today. Suitable radioisotopes include, for example, yttrium^{90}(^{90}Y), indium^{111} (^{111}In), ^{131}I, ^{99}mTc, radiosilver-111, radiosilver-199, and Bismuth^{213}. The linkage of radioisotopes to binding proteins may be performed with, for example, conventional bifunctional chelates. Since silver is monovalent, for radiosilver-111 and radiosilver-199 linkage, sulphur-based linkers may be used (Hazra et al. (1994) Cell Biophys. 24-25:1-7). Linkage of silver radioisotopes may involve reducing the immunoglobulin with ascorbic acid. Furthermore, tiuxetan is an MX-DTPA linker chelator attached to ibritumomab to form ibritumomab tiuxetan (Zevalin) (Witzig (2001) Cancer Chemother. Pharmacol. 48 (Suppl 1):91-95). Ibritumomab tiuxetan can react with radioisotypes such as indium^{111} (^{111}In) or ^{90}Y to form ^{111}In-ibritumomab tiuxetan and ^{90}Y-ibritumomab tiuxetan, respectively.

[0078] The binding proteins described herein, particularly when used to treat cancer, can be conjugated with toxic chemotherapeutic drugs such as maytansinoids, (Hamann et al. (2002) Bioconjug. Chem. 13:40-46), geldanamycinoids (Mandler et al. (2000) J. Natl. Cancer Inst. 92:1549-1551) and maytansinoids" for example, the maytansinoid drug, DM1 (Liu et al. (1996) Proc. Natl. Acad. Sci. US 93:8618-8623). Linkers that release the drugs under acidic or reducing conditions or upon exposure to specific proteases may be employed with this technology. A binding protein may be conjugated as described in the art.

[0079] A binding protein can be conjugated to auristatin-PE. Auristatin-PE, *e.g.*, is an antimicrotubule agent that is a structural modification of the marine, shell-less mollusk peptide constituent dolastatin 10. Auristatin-PE has both anti-tumor activity and anti-tumor vascular activity (Otani et al. (2000) Jpn. J. Cancer Res. 91:837-44). For example, auristatin-PE inhibits cell growth and induces cell cycle arrest and apoptosis in pancreatic cancer cell lines (Li et al. (1999) Int. J. Mol. Med. 3:647-53). Accordingly, to specifically target the anti-tumor activity and anti-tumor vascular activities of auristatin-PE to particular tumors, auristatin-PE may be conjugated to a binding protein as described herein.

[0080] The pharmaceutical compositions described herein also can contain at least one further active agent. Examples of further active agents include antibodies or low molecular weight inhibitors of other receptor protein kinases, such as IGFR-1 and c-met, receptor ligands such as vascular endothelial factor (VEGF), cytotoxic agents such as doxorubicin, cisplatin or carboplatin, cytokines, or anti-neoplastic agents. Many anti-neoplastic agents are known in the art. An anti-neoplastic agent can be selected from the group of therapeutic proteins including, but not limited to, antibodies and immunomodulatory proteins. An anti-neoplastic agent can be selected from the group of small molecule inhibitors and chemotherapeutic agents consisting of mitotic inhibitors, kinase inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, histone deacetylase inhibitors, anti-survival agents, biological response modifiers, anti-hormones (*e.g.*, anti-androgens), microtubule stimulants, anthracyclins, and anti-angiogenesis agents. When the anti-neoplastic agent is radiation, treatment can be achieved either with an internal source (*e.g.*, brachytherapy) or an external source (*e.g.*, external beam radiation therapy). The one or more further active agent(s) can be administered with the HER3-binding agent and the second agent either simultaneously or separately, in a single formulation or in individual (separate) formulations for each active agent.

[0081] The pharmaceutical compositions described herein can be especially useful for diagnosis, prevention, or treatment of a hyperproliferative disease. The hyperproliferative disease can be associated with increased HER family signal transduction. In particular, the disease can be associated with increased HER-3 phosphorylation, increased complex formation between HER-3 and other members of the HER family, increased PI_3 kinase activity, increased c-jun terminal kinase activity and/or AKT activity, increased ERK2 and/or PYK2 activity, or any combination thereof. The hyperprolif-

erative disease can be, for example, selected from the group consisting of breast cancer, gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, kidney cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma, or other HER-3 expressing or overexpressing cancers, and the formation of tumor metastases.

[0082] Pharmaceutical compositions can be formulated by mixing one or more active agents with one or more physiologically acceptable carriers, diluents, and/or adjuvants, and optionally other agents that are usually incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A pharmaceutical composition can be formulated, *e.g.,* in lyophilized formulations, aqueous solutions, dispersions, or solid preparations, such as tablets, dragees or capsules. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (18th ed, Mack Publishing Company, Easton, PA (1990)), particularly Chapter 87 by Block, Lawrence, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies as described herein, provided that the active agent in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See,* also, Baldrick (2000) Regul. Toxicol. Pharmacol. 32:210-218; Wang (2000) Int. J. Pharm. 203:1-60; Charman (2000) J. Pharm. Sci. 89:967-978; and Powell et al. (1998) PDA J. Pharm. Sci. Technol. 52:238-311), and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[0083] This document also pertains to the use of at least one agent (*e.g.*, an isolated HER-3 binding protein) as described herein, and at least one other active agent (*e.g.*, an agent that binds to another HER family member or a chemotherapeutic compound) in admixture with pharmaceutically acceptable carriers, diluents and/or adjuvants, for the manufacture of a pharmaceutical composition for diagnosis, prevention or treatment of a hyperproliferative disease (*e.g.,* a disease associated with HER-3). The pharmaceutical composition can be a pharmaceutical composition as described herein, and the hyperproliferative disease can be a hyperproliferative disease as described herein.

[0084] Methods for formulating and subsequently administering therapeutic compositions are well known to those skilled in the art. Dosing generally is dependent on the severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Persons of ordinary skill in the art routinely determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages can vary depending on the relative potency of individual polypeptides, and can generally be estimated based on EC_{50} found to be effective in *in vitro* and *in vivo* animal models. Typically, dosage is from 0.1 μg to 100 mg per kg of body weight, and may be given once or more daily, biweekly, weekly, monthly, or even less often. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state.

[0085] Pharmaceutical compositions can be administered by a number of methods, depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be, for example, topical (*e.g.*, transdermal, sublingual, ophthalmic, or intranasal); pulmonary (*e.g.*, by inhalation or insufflation of powders or aerosols); oral; or parenteral (*e.g.*, by subcutaneous, intrathecal, intraventricular, intramuscular, or intraperitoneal injection, or by intravenous drip). Administration can be rapid (*e.g.,* by injection) or can occur over a period of time (*e.g.,* by slow infusion or administration of slow release formulations). For treating tissues in the central nervous system, HER-3 binding proteins can be administered by injection or infusion into the cerebrospinal fluid, typically with one or more agents capable of promoting penetration of the polypeptides across the blood-brain barrier.

[0086] Compositions and formulations for parenteral, intrathecal or intraventricular administration can include sterile aqueous solutions, which also can contain buffers, diluents and other suitable additives (*e.g.*, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers).

[0087] Pharmaceutical compositions include, without limitation, solutions, emulsions, aqueous suspensions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, for example, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Emulsions are often biphasic systems comprising of two immiscible liquid phases intimately mixed and dispersed with each other; in general, emulsions are either of the water-in-oil (w/o) or oil-in-water (o/w) variety. Emulsion formulations have been widely used for oral delivery of therapeutics due to their ease of formulation and efficacy of solubilization, absorption, and bioavailability.

[0088] HER binding agents can further encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, this document provides pharmaceutically acceptable salts of small molecules and polypeptides, prodrugs and pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form and is converted to an active form (*i.e.,* drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. The term "pharmaceutically acceptable salts" refers to

physiologically and pharmaceutically acceptable salts of the polypeptides provided herein (*i.e.*, salts that retain the desired biological activity of the parent polypeptide without imparting undesired toxicological effects). Examples of pharmaceutically acceptable salts include, but are not limited to, salts formed with cations (*e.g.*, sodium, potassium, calcium, or polyamines such as spermine); acid addition salts formed with inorganic acids (*e.g.*, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, or nitric acid); and salts formed with organic acids (*e.g.*, acetic acid, citric acid, oxalic acid, palmitic acid, or fumaric acid).

[0089] Described herein are pharmaceutical compositions containing (a) one or more HER-3 binding agents; (b) one or more agents that bind to another HER family member; and (c) one or more other agents that function by a different mechanism. For example, one or more agents of (c) are exchangeable with those of (b); anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, can be included in compositions. Other non-polypeptide agents (*e.g.*, chemotherapeutic agents) also are within the scope of this document. Such combined compounds can be used together or sequentially.

[0090] Compositions additionally can contain other adjunct components conventionally found in pharmaceutical compositions. Thus, the compositions also can include compatible, pharmaceutically active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or additional materials useful in physically formulating various dosage forms of the compositions provided herein, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. Furthermore, the composition can be mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings, and aromatic substances. When added, however, such materials should not unduly interfere with the biological activities of the polypeptide components within the compositions provided herein. The formulations can be sterilized if desired.

[0091] The pharmaceutical formulations, which can be presented conveniently in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients (*e.g.*, the HER family binding agents provided herein) with the desired pharmaceutical carrier(s) or excipient(s). Typically, the formulations can be prepared by uniformly and bringing the active ingredients into intimate association with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product. Formulations can be sterilized if desired, provided that the method of sterilization does not interfere with the effectiveness of the polypeptide contained in the formulation.

[0092] The compositions described herein can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions also can be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions further can contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol, and/or dextran. Suspensions also can contain stabilizers.

[0093] HER binding proteins can be combined with packaging material and sold as kits for treating HER-3 associated diseases. Components and methods for producing articles of manufacture are well known. The articles of manufacture may combine one or more of the polypeptides and compounds set out in the above sections. In addition, the article of manufacture further may include, for example, buffers or other control reagents for reducing or monitoring reduced immune complex formation. Instructions describing how the polypeptides are effective for treating HER-3 associated diseases can be included in such kits. Any of the first agents, the second agents and additional agents could be delivered in nanoparticle(s) or liposome(s), or any other suitable form(s)

6. Methods

[0094] This document also describes methods for treating or preventing diseases and conditions associated with expression of HER-3. For example, a method can include contacting a subject or a biological sample from a subject (*e.g.*, a mammal such as a human) with a HER-3 binding protein in combination with a second agent as described herein. The sample may be a cell that shows expression of HER-3, such as a tumor cell, a blood sample or another suitable sample. The contacting can occur *in vivo,* such as when a composition containing a HER-3 binding agent and a second agent that binds to another member of the HER family is administered to a subject in need thereof. The diseases or conditions associated with expression of HER-3 that can be treated using the methods described herein include, for example, hyperproliferative diseases such as breast cancer, gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, kidney cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma, renal cancer, metastatic breast cancer, non-small cell lung cancer, epidermoid carcinoma, fibrosarcoma, melanoma, nasopharyngeal carcinoma, squamous cell carcinoma, and other HER-3-positive, -expressing or - overexpressing cancers.

[0095] The term "treatment or prevention," when used herein, refers to both therapeutic treatment and prophylactic or preventative measures, which can be used to prevent, slow, or lessen the effects of the targeted pathologic condition

or disorder. Those in need of prevention or treatment can include those already having the disorder, as well as those who may be likely to develop the disorder, or those in whom the disorder is to be prevented. The patient in need of prevention or treatment can be a mammalian patient (*i.e.,* any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc.) In some embodiments, the patient in need of treatment is a human patient.

[0096] Methods for preventing or treating diseases or conditions associated with expression of HER-3 in a patient in need thereof can include administering to the patient effective amounts of at least one HER-3 binding agent as described herein and at least one other agent against another HER family member, or a chemotherapeutic compound (*e.g.*, at least one of the "additional/further" agents described above). Such treatment can, for example, inhibit abnormal cell growth, migration or invasion. The agent against HER-3 and the at least one other agent can be administered simultaneously (*e.g.*, when they are contained in the same composition, or by admixture into a common i.v. bag), or separately (*e.g.*, sequentially). The diseases or conditions associated with the expression of HER-3 that can be treated using the methods provided herein include, for example, the hyperproliferative diseases listed herein. The patient in need of prevention or treatment can be a mammal (*e.g.*, a human, a domestic or farm animal, or a zoo, sport, or pet animal such as a dog, cat, cow, horse, sheep, pig, goat, or rabbit). In some cases, the patient is a human patient.

[0097] As used herein, the term "effective amount" is an amount of an agent that results in a decrease or stabilization in one or more symptoms or clinical characteristics of the HER-3 associated condition being treated. For example, administration of an effective amount of a composition as described herein can result in slowing of tumor growth progression, in decreased tumor size, or in decreased activation of HER-3 or HER-3-responsive biomarkers (e.g., Akt, HER-2, ERK, or EGF-R). The slowing or decrease can be any reduction as compared to a previous value (*e.g.*, a 5%, 10%, 20%, 25%, or more than 25% reduction in symptom or characteristic). An "effective amount" can result in stable disease.

[0098] In addition to classical modes of administration of potential binding protein therapeutics, *e.g.*, via the above mentioned formulations, newly developed modalities of administration may also be useful. For example, local administration of ^{131}I-labeled monoclonal antibody for treatment of primary brain tumors after surgical resection has been reported. Additionally, direct stereotactic intracerebral injection of monoclonal antibodies and their fragments is also being studied clinically and pre-clinically. Intracarotid hyperosmolar perfusion is an experimental strategy to target primary brain malignancy with drug conjugated human monoclonal antibodies.

[0099] As described above, the dose of the agents administered can depend on a variety of factors. These include, for example, the nature of the agents, the tumor type, and the route of administration. It should be emphasized that the present methods are not limited to any particular doses. Methods for determining suitable doses are known in the art, and include those described in the Examples herein.

[0100] Depending on the type and severity of the condition to be treated, up to about 20 mg/kg of each HER binding antibody can be administered to a patient in need thereof, *e.g.,* by one or more separate administrations or by continuous infusion. A typical daily dosage might range from about 1 μg/day to about 100 mg/day or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition to be treated, the treatment can be sustained until a desired suppression of disease symptoms occurs.

[0101] A method as described herein can include analyzing a particular marker *(e.g.,* HER-3) in a biological sample from a subject to determine whether the subject has a disease associated with HER-3 expression. Such methods can be used to select subjects having diseases associated with HER-3. In such methods, the analyzing step can be done prior to the step of administration, as such screening of patients may avoid treatments that are not likely to be effective. Thus, in some cases, the methods described herein can further include detecting HER-3 antigen in or on a cell, for determination of HER-3 antigen concentration in patients suffering from a hyperproliferative disease as mentioned above, or for staging of a hyperproliferative disease in a patient. In order to stage the progression of a hyperproliferative disease in a subject under study, or to characterize the response of the subject to a course of therapy, a sample of blood can be taken from the subject and the concentration of the HER-3 antigen present in the sample can be determined. The concentration so obtained can be used to identify in which range of concentrations the value falls. The range so identified can be correlated with a stage of progression or a stage of therapy identified in the various populations of diagnosed subjects, thereby providing a stage for the subject under study. A biopsy of the disease, *e.g.*, cancerous, tissue obtained from the patient also can be used assess the amount of HER-3 antigen present. The amount of HER-3 antigen present in the disease tissue may be assessed using, for example, immunohistochemistry, ELISA, or antibody array using HER-3 antibodies as described herein. Other parameters of diagnostic interest are the dimerization state as well as the dimerization partners of the HER-3 protein and the activation state of it and its partners. Protein analytical methods to determine those parameters are well known in the art and are among others western blot and immunoprecipitation techniques, FACS analysis, chemical crosslinking, bioluminescence resonance energy transfer (BRET), fluorescence resonance energy transfer (FRET) and the like (*e.g.,* Price et al. (2002) Methods Mol. Biol. 218:255-268, or eTag technology (WO 05/03707, WO 04/091384, and WO 04/011900).

[0102] In some cases, a method as described herein can include one or more steps for monitoring the therapeutic

outcome of the treatment. For example, a subject can be monitored for symptoms of their disease, to determine whether a reduction in symptoms has occurred. The subject also can be monitored, for example, for potential side effects of the treatment. The monitoring can be done after the administration step, and, in some embodiments, can be done multiple times (*e.g.*, between administrations, if dosages are given more than once). Such methods can be used to assess efficacy and safety of the treatment methods described herein, for example.

[0103] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

EXAMPLE 1: HER-3 Antigen and Cell Line Preparation

[0104] Recombinant HER-3 proteins were prepared. The extracellular domain of HER-3 (ECD) cDNA was cloned by polymerase chain reaction (PCR) from pcDNA3-HER-3 (expression vector with full length human HER-3, Wallasch et al. (1995) EMBO J. 14:4267-4275) with primers based on the sequence of HER-3 (GeneBank Accession No. NM_001982): Forward primer: 5'-CGGGATCCATGTCCTAGCCTAGGGGC-3' (SEQ ID NO: 233); Reverse primer: 5'-GCTCTAGATTAATGATGATGATGATGATG TTGTCCTAAACAGTCTTG-3' (SEQ ID NO: 234).

[0105] The PCR product was digested with BamH1 and XbaI and ligated into pcDNA3 (Invitrogen) digested with BamH1 and XbaI. Plasmids were transfected into HEK293 cells using a $CaPO_4$ method. The HER-3-HIS fusion protein was purified from harvested conditioned media via Ni-NTA affinity chromatography.

[0106] RatI HER-3 cells were generated by retroviral gene transfer. Briefly, GP+E 86 cells ($3x10^5$) were seeded on a 60 mm culture disc and transfected with 2 μg/ml plXSN vector or plXSN-HER-3 cDNA (C. Wallasch, PhD Thesis, Max-Planck Institute of Biochemistry, Martinsried, Germany) using the calcium phosphate method. After 24 hours, the medium was replaced with fresh medium and the GP+E 86 cells were incubated for 4-8 hours. Subconfluent Rat1 cells ($2x10^5$ cells per 6 cm dish) were then incubated with supernatants of GP+E 86 cells releasing high titer pLXSN or pLXSN-HER-3, p virus (>1 X 10^6 G418 c.f.u./ml; m.o.i. of 10) for 4-12 hours in the presence of Polybrene (4 mg/ml; Aldrich). After changing the medium, selection of RatI cells with G418 was started. Usually, stable clones were picked after selection for 21 days.

EXAMPLE 2: HER-3 Expression in Human Cancer Cell Lines

[0107] HER-3 expression was quantified in a panel of human cancer cell lines to elucidate the role of HER-3 in human cancer formation. Cancer cell lines were grown as recommended by the ATCC. In detail, 10^5 cells were harvested with 10 mM EDTA in PBS, washed once with FACS buffer (PBS, 3 % FCS, 0.4 % azide) and seeded on a 96-well round bottom plate. The cells were spun for 3 minutes at 1000 rpm to remove supernatant and then resuspended with □-HER-3 antibody 2D1D12 (WO03013602) (3 μg/ml). Cell suspensions were incubated on ice for 1 hour, washed twice with FACS buffer, and resuspended with secondary antibody (100 μl/well) donkey-anti-human-PE (Jackson) diluted 1:50 in FACS buffer. The cell suspensions were incubated on ice and in the dark for 30 minutes, washed twice with FACS buffer and analyzed (FACS, Beckman Coulter). HER-3 was expressed in a variety of human cancer cell lines, including various breast, colon, epidermoid, melanoma, nasopharynx, ovarian, pancreas, and prostate cell lines. *See,* the figures of US Publication No. 20080124345.

EXAMPLE 3: Immunization and Titering

[0108] The HER-3 ECD protein that was prepared as described in Example 1 and C32 cells (Human melanoma; ATCC #CRL-1585) were used as antigen. Monoclonal antibodies against HER-3 were developed by sequentially immunizing XENOMOUSE® mice (strains XMG1 and XMG4; Abgenix, Inc., Fremont, CA). XENOMOUSE® animals were immunized via the footpad for all injections. The total volume of each injection was 50 μl per mouse, 25 μl per footpad.

[0109] For cohort #1 (10 XMG1 mice), the initial immunization was with 10 μg of HER-3 ECD protein admixed 1:1 (v/v) with TITERMAX GOLD® (Sigma, Oakville, ON) per mouse. The subsequent five boosts were made with 10 μg of HER-3 ECD protein admixed 1:1 (v/v) with 100 μg alum gel (Sigma, Oakville, ON) in pyrogen-free D-PBS. The sixth boost consisted of 10 μg of HER-3 ECD protein admixed 1:1 (v/v) with TITERMAX GOLD®. The seventh injection consisted of 10 μg of HER-3 ECD protein admixed 1:1 v/v with 100 μg alum gel. A final boost was made with 10 μg HER-3 ECD protein in pyrogen-free DPBS, without adjuvant. The XENOMOUSE® mice were immunized on days 0, 4, 7, 11, 15, 20, 24, and 29 for this protocol, and fusions were performed on day 33. The two bleeds were made through Retro-Orbital Bleed procedure on day 13 after the fourth boost and on day 19 after the sixth boost. There was no cohort #2. For Cohort #3 (10 XMG1 mice) and Cohort #4 (10 XMG4 mice), the first injection was with 10^7 C32 cells in pyrogen-free Dulbecco's PBS (DPBS) admixed 1:1 (v/v) with TITERMAX GOLD® per mouse. The next four boosts were with

10^7 C32 cells in pyrogen-free DPBS, admixed with 25 μg of Adju-Phos and 10 μg CpG per mouse. The sixth boost was with 10^7 C32 cells in pyrogen-free DPBS, admixed 1:1 (v/v) with TITERMAX GOLD® per mouse. The seventh, eighth, and ninth boosts were with 10^7 C32 cells in pyrogen-free DPBS, admixed with 25 μg of Adju-Phos and 10 μg CpG per mouse. The tenth to fourteenth boosts were with 5 μg of HER-3 ECD protein in pyrogen-free DPBS, admixed with 25 μg of Adju-Phos and 10 μg CpG per mouse. A final boost consisted of 5 μg of HER-3 ECD protein in pyrogen-free DPBS, without adjuvant. For both Cohorts #3 and #4, the mice were immunized on days 0, 3, 7, 11, 14, 17, 21, 24, 28, 33, 35, 38, 42 and 45, and fusions were performed on day 49. The three bleeds were made through Retro-Orbital Bleed procedure on day 12 after the fourth boost, on day 19 after the sixth boost, and on day 40 after twelfth boost.

[0110] Selection of animals for harvest by titer: For cohort #1, anti-HER-3 antibody titers in the serum from immunized mice were determined by ELISA against HER-3 ECD protein. The specific titer of each XENOMOUSE® animal was determined from the optical density at 650 nm, and is shown in TABLE 1 below. The titer value is the reciprocal of the greatest dilution of sera with an OD reading two-fold that of background. Therefore, the higher the number, the greater the humoral immune response to HER-3 ECD.

TABLE 1

Cohort #1, XMG1		
Mouse ID	**After 4 injections**	**After 6 injections**
P3421	8,000	11,000
P3422	850	2,600
P3423	2,700	5,200
P3424	3,200	9,100
P3425	5,400	2,500
P3426	700	1,500
P3427	5,800	7,000
P3428	3,900	4,300
P3429	2,200	2,500
P34210	600	850
NC	250	175
PC	377,000	311,000
NC	mAb IL-8, D39.2.1	
PC	xHER-3-2D1D12	

[0111] For cohorts #3 and #4, anti-HER-3 antibody titers in the serum from immunized mice were determined by FACS using Rat1/HER-3 (antigen positive cell line) cells and Rat1/pLSXN (antigen negative cell line) cells. Data are shown in TABLES 2 and 3, and are presented as geometric mean (GeoMean) fluorescent intensity of cell anti-HER-3 cell staining by serial dilutions of serum samples.

TABLE 2

Cohort #3, XMG1					
Mouse ID	**Sample**	**After 6 injections**		**After 12 injections**	
		pos cells GeoMean	neg cells GeoMean	pos cells GeoMean	neg cells GeoMean
Q832-1	1:50	9	10	11	10
	1:250	6	9	6	6
	1:1250	6	7	4	4
Q832-2	1:50	8	10	29	42
	1:250	7	8	11	11

(continued)

Cohort #3, XMG1					
Mouse ID	**Sample**	**After 6 injections**		**After 12 injections**	
		pos cells GeoMean	neg cells GeoMean	pos cells GeoMean	neg cells GeoMean
	1:1250	5	6	6	5
Q832-3	1:50	7	12	11	9
	1:250	5	7	5	5
	1:1250	5	5	4	4
Q832-4	1:50	6	10	9	9
	1:250	6	6	5	5
	1:1250	5	5	4	4
Q832-5	1:50	11	11	17	13
	1:250	10	9	7	6
	1:1250	6	8	5	4
Q832-6	1:50	7	11	15	14
	1:250	7	7	7	6
	1:1250	5	6	6	4
Q832-7	1:50	8	11	7	15
	1:250	6	7	5	5
	1:1250	5	5	4	4
Q832-8	1:50	7	8	11	20
	1:250	6	6	7	8
	1:1250	5	5	5	4
Q832-9	1:50	7	12	15	16
	1:250	6	8	6	5
	1:1250	6	6	4	4
Q832-10	1:50	8	13	34	38
	1:250	6	8	9	8
	1:1250	6	6	5	4

TABLE 3

Cohort #4, XMG4					
Mouse	**Sample**	**After 6 injections**		**After 12 injections**	
		pos cells GeoMean	neg cells GeoMean	pos cells GeoMean	neg cells GeoMean
Q856-1	1:50	4	6	91	44
	1:250	4	5	32	18
	1:1250	4	4	19	10
Q856-2	1:50	4	8	148	54

(continued)

Cohort #4, XMG4					
Mouse	**Sample**	**After 6 injections**		**After 12 injections**	
		pos cells GeoMean	neg cells GeoMean	pos cells GeoMean	neg cells GeoMean
	1:250	4	5	89	23
	1:1250	4	4	42	9
Q856-3	1:50	4	5	72	14
	1:250	4	4	28	6
	1:1250	4	4	18	4
Q856-4	1:50	4	5	11	49
	1:250	4	5	10	17
	1:1250	4	4	8	7
Q856-5	1:50	4	4	74	20
	1:250	4	4	30	14
	1:1250	4	4	16	6
Q856-6	1:50	4	5	86	21
	1:250	4	4	32	10
	1:1250	4	4	16	5
Q856-7	1:50	5	6	74	32
	1:250	4	5	32	14
	1:1250	4	4	16	6
Q856-8	1:50	4	5	106	14
	1:250	4	4	45	6
	1:1250	4	4	22	4
Q856-9	1:50	5	6	53	22
	1:250	4	4	17	11
	1:1250	4	4	11	5
Q856-10	1:50	4	5	72	53
	1:250	4	4	26	17
	1:1250	4	4	15	7

EXAMPLE 4: Recovery of Lymphocytes, B-Cell Isolations, Fusions and Generation of Hybridomas

[0112] Immunized mice were sacrificed and the lymph nodes were harvested and pooled from each cohort. The lymphoid cells were dissociated by grinding in DMEM to release the cells from the tissues, and the cells were suspended in DMEM. The cells were counted, and 0.9 ml DMEM per 100 million lymphocytes was added to the cell pellet to resuspend the cells gently but completely. Using 100 μl of CD90+ magnetic beads per 100 million cells, the cells were labeled by incubating the cells with the magnetic beads at 4°C for 15 minutes. The magnetically-labeled cell suspension containing up to 10^8 positive cells (or up to $2x10^9$ total cells) was loaded onto a LS+ column and the column washed with DMEM. The total effluent was collected as the CD90-negative fraction (most of these cells were expected to be B cells).

[0113] The fusion was performed by mixing washed enriched B cells from above and nonsecretory myeloma P3X63Ag8.653 cells purchased from ATCC (Cat. No. CRL 1580) (Kearney et al. (1979) J. Immunol. 123:1548-1550) at a ratio of 1:1. The cell mixture was gently pelleted by centrifugation at 800 g. After complete removal of the supernatant,

the cells were treated with 2 to 4 ml of pronase solution (CalBiochem, Cat. No. 53702; 0.5 mg/ml in PBS) for no more than 2 minutes. Then 3 to 5 ml of FBS was added to stop the enzyme activity, and the suspension was adjusted to 40 ml total volume using electro cell fusion solution, ECFS (0.3 M sucrose, Sigma, Cat. No. S7903, 0.1 mM magnesium acetate, Sigma, Cat. No. M2545, 0.1 mM calcium acetate, Sigma, Cat. No. C4705). The supernatant was removed after centrifugation and the cells were resuspended in 40 ml ECFS. This wash step was repeated and the cells again were resuspended in ECFS to a concentration of $2x10^6$ cells/ml.

[0114] Electro-cell fusion was performed using a fusion generator, model ECM2001, Genetronic, Inc., San Diego, CA. The fusion chamber size was 2.0 ml, and the following instrument settings were used: Alignment conditions: voltage: 50 V, time: 50 seconds; membrane breaking: voltage: 3000 V, time: 30 μseconds; post-fusion holding time: 3 seconds.

[0115] After ECF, the cell suspensions were removed from the fusion chamber under sterile conditions and transferred into a sterile tube containing the same volume of Hybridoma Culture Medium (DMEM (JRH Biosciences), 15 % FBS (Hyclone), supplemented with L-glutamine, pen/strep, OPI (oxaloacetate, pyruvate, bovine insulin) (all from Sigma) and IL-6 (Boehringer Mannheim). The cells were incubated for 15 to 30 minutes at 37°C, and then centrifuged at 400 g for five minutes. The cells were gently resuspended in a small volume of Hybridoma Selection Medium (Hybridoma Culture Medium supplemented with 0.5x HA (Sigma, Cat. No. A9666)), and the volume was adjusted appropriately with more Hybridoma Selection Medium, based on a final plating of $5x10^6$ B cells total per 96-well plate and 200 μl per well. The cells were mixed gently and pipetted into 96-well plates and allowed to grow. On day 7 or 10, half of the medium was removed, and the cells were re-fed with Hybridoma Selection Medium.

EXAMPLE 5: Selection of Candidate Antibodies by ELISA

[0116] After 14 days of culture, primary screening of hybridoma supernatants from the cohort #1 (mice in cohort one were split arbitrarily into fusion #1 and #2) for HER-3-specific antibodies was performed by ELISA using purified his-tagged HER-3 ECD and counter-screening against an irrelevant his-tagged protein by ELISA using goat anti-huIgGFc-HRP (Caltag Inc., Cat. No. H10507, using concentration was 1:2000 dilution) to detect human IgG binding to HER-3 ECD immobilized on ELISA plates. The old culture supernatants from positive hybridoma cells growth wells based on primary screen were removed, and the HER-3 positive hybridoma cells were suspended with fresh hybridoma culture medium and were transferred to 24-well plates. After 2 days in culture, these supernatants were used for a secondary confirmation screen. In the secondary confirmation screen for HER-3 specific fully human IgGk antibodies, the positives in the first screening were screened by ELISA with two sets of detective antibodies: goat anti-huIgGFc-HRP (Caltag Inc., Cat. No. H10507, using a 1:2000 dilution) for human gamma chain detection, and goat anti-hIg kappa-HRP (Southern Biotechnology, Cat. No. 2060-05) for human kappa light chain detection. From cohort #1, 91 fully human IgG/kappa HER-3 specific monoclonal antibodies were generated.

EXAMPLE 6: Selection of Candidate Antibodies by FMAT/FACS

[0117] After 14 days of culture, hybridoma supernatants from the cohorts #3 and #4 (fusions #3 and #4) were screened for HER-3-specific monoclonal antibodies by FMAT. In the primary screen, hybridoma supernatants at 1:10 final dilution were incubated with Rat1-HER-3 cells expressing human HER-3 and 400 ng/ml Cy5-conjugated Goat F(ab')2 anti-human IgG, Fc-specific antibody (Jackson ImmunoResearch, Cat. No. 109-176-098) at room temperature for 6 hours. The binding of antibodies and detection antibodies to cells were measured by FMAT (Applied Biosystems). Non-specific binding of antibodies to the cells was determined by their binding to parental Rat1 cells. A total of 420 hybridomas producing HER-3-specific antibodies were selected from the primary screen of fusion #3. The supernatants from these expanded cultures were tested again using the same FMAT protocol, and 262 of them were confirmed to bind specifically to HER-3 expressing cells. A total of 193 hybridomas producing HER-3 specific antibodies were selected from the primary screen of fusion #4. The supernatants from these expanded cultures were tested by FACS, and 138 of them were confirmed to bind specifically to cells expressing HER-3. In the FACS confirmation assay, Rat1-XHER-3 cells and parental Rat1 cells (as negative control) were incubated with hybridoma supernatants at 1:2 dilution for 1 hour at 40°C in PBS containing 2 % FBS. Following washing with PBS, the binding of antibodies to the cells was detected by 2.5 μg/ml Cy5-conjugated Goat F(ab')2 anti-human IgG, Fc-specific antibody (JIR#109-176-098) and 5 μg/ml PE-conjugated Goat F(ab')2 anti-human kappa-specific antibody (SB# 2063-09). After removing unbound antibodies by washing with PBS, the cells were fixed by cytofix (BD# 51-2090KZ) at 1:4 dilution and analyzed by FACSCalibur.

EXAMPLE 7: Selection of Hybridomas for Cloning

[0118] Antibodies from cohort #1 were selected for hybridoma cloning based on specificity for HER-3 over HER1 (EGF-R), HER-2 and HER-4 in ELISA using purified recombinant extra-cellular domains (available from, for example, R&D Biosystems, Minneapolis, MN), FACS-based analysis of human tumor cell lines expressing different HER family

members, and a > 5-time increase in mean fluorescent intensity in FACS staining for HER-3 positive cells over background. Based on these criteria, a total of 23 hybridoma lines were selected for cloning by limiting dilution cell plating.

[0119] Antibodies from cohorts 3 and 4 were selected for hybridoma cloning based on specificity for HER-3 over HER-1 (EGF-R), HER-2 and HER-4 plus three other criteria. The first criterion was an ELISA screen for antibodies with epitopes contained within the L2 domain of HER-3 (*see,* Example 8 below).

[0120] The second criterion was neutralization of binding of biotinylated heregulin-alpha to HER-3 expressing cells in a FACS based assay. SKBR-3 cells were harvested, washed in culture medium, pelleted via centrifugation and resuspended in culture medium. Resuspended cells were aliquoted into 96-well plates. The plates were centrifuged to pellet the cells. Test antibodies in exhaust hybridoma supernatants were added at 25 μl/well and incubated for 1 hour on ice to allow antibody binding. Fifty μl of a 10 nM heregulin-alpha (R&D Biosystems) solution was added to each well for a final concentration of 5 nM and incubated on ice for 1.5 hours. Cells were washed in 150 μl PBS, pelleted by centrifugation and the supernatant removed. Cells were resuspended in 50 μl of goat anti-HRG-alpha polyclonal antibody at 10 μg/ml and incubated for 45 minutes on ice. Cells were washed in 200 μl PBS, pelleted by centrifugation, and the supernatant was removed. Fifty μl of a solution of rabbit Cy5-labeled anti-goat polyclonal antibody at 5 μg/ml plus 7AAD at 10 μg/ml was added and incubated on ice for 15 minutes. Cells were washed in 200 μl PBS, pelleted by centrifugation and the supernatant removed. The cells were resuspended in 100 μl of FACS buffer and read in the FACS. Test HER-3 antibodies that reduced binding of heregulin-alpha were those that had lowest fluorescence intensity. As positive controls, 1:5 serial dilutions from 10,000 ng/ml to 16 ng/ml of a mouse HER-3 mAb (105.5) or the human IgG1 HER-3 mAb, U1-49 was used. Negative controls were heregulin-alpha alone, cells alone, goat anti-heregulin-alpha polyclonal antibody alone and rabbit Cy5-labeled anti-goat polyclonal antibody alone.

[0121] The third criterion was relative ranking for affinity and/or higher relative mean fluorescence intensity in FACS using HER-3 expressing cell lines. Relative ranking for affinity was performed by normalizing HER-3-specific antibody concentrations and plotting versus data from limiting antigen ELISA as follows.

[0122] *Normalization of antigen specific antibody concentrations using high antigen ELISA:* Using an ELISA method, supernatants for concentration of antigen specific antibody were normalized. Using two anti-HER-3 human IgG1 antibodies from cohort 1 of known concentration titrated in parallel, a standard curve was generated and the amount of antigen specific antibody in the test hybridoma supernatants from cohorts 3 and 4 were compared to the standard. In this way, the concentration of human HER-3 IgG antibody in each hybridoma culture was estimated.

[0123] Neutravidin plates were made by coating neutravidin at 8 μg/ml in 1XPBS/0.05% sodium azide on Costar 3368 medium binding plates at 50 μl/well with overnight incubation at 4°C. The next day the plates were blocked with 1XPBS/1% skim milk. Photobiotinylated his-tagged-HER-3 ECD at 500 ng/ml in 1XPBS/1% skim milk was bound to the neutravidin plates by incubating for 1 hour at room temperature. Hybridoma supernatant, serially diluted 1:2.5 from a starting dilution of 1:31 to a final dilution of 1:7568 in1XPBS/1% skim milk/0.05% azide, was added at 50 μl/well, and then incubated for 20 hours at room temperature. Serial dilutions were used to ensure obtaining OD readings for each unknown in the linear range of the assay. Next, a secondary detection antibody, goat anti human IgG Fc HRP at 400 ng/ml in 1XPBX/1% skim milk was added at 50 μl/well. After 1 hour at room temperature, the plates were again washed 5 times with water and 50 μl of one-component TMB substrate were added to each well. The reaction was stopped after 30 minutes by addition of 50 μl 1M hydrochloric acid to each well and the plates were read at wavelength 450 nm. A standard curve was generated from the two IgG1 HER-3 mAbs from cohort #1, serially diluted at 1:2 from 1000 ng/ml to 0.06 ng/ml and assessed in ELISA using the above protocol. For each unknown, OD readings in the linear range of the assay were used to estimate the concentration of human HER-3 IgG in each sample.

[0124] The limited antigen analysis is a method that affinity ranks the antigen-specific antibodies prepared in B-cell culture supernatants relative to all other antigen-specific antibodies. In the presence of a very low coating of antigen, only the highest affinity antibodies should be able to bind to any detectable level at equilibrium. (*See, e.g.,* PCT Publication No. WO 03048730A2). In this instance, two mAbs from cohort #1, both of known concentration and known KD, were used as benchmarks in the assay.

[0125] Neutravidin plates were made by coating neutravidin at 8 μg/ml in 1XPBS/0.05% sodium azide on Costar 3368 medium binding plates at 50 μl/well with overnight incubation at 4°C. The next day the plates were blocked with 1XPBS/1% skim milk. Biotinylated his-tagged-HER-3 ECD (50 μl/well) was bound to 96-well neutravidin plates at five concentrations: 125, 62.5, 31.2, 15.6, and 7.8 ng/ml in 1XPBS/1% skim milk for 1 hour at room temperature. Each plate was washed 5 times with water. Hybridoma supernatants diluted 1:31 in 1XPBS/1%skim milk/0.05% azide were added at 50 ul/well. After 20 hours incubation at room temperature on a shaker, the plates were again washed 5 times with dH_2O. Next, a secondary detection antibody, goat anti human IgG Fc HRP (Horse Radish Peroxidase) at 400 ng/ml in 1XPBS/1% skim milk was added at 50 μl/well. After 1 hour at room temperature, the plates were again washed 5 times with dH_2O and 50 μL of one-component TMB substrate were added to each well. The reaction was stopped after 30 minutes by addition of 50 μl of 1M hydrochloric acid to each well and the plates were read at wavelength 450 nm. OD readings from an antigen concentration that yielded OD values in the linear range were used in for data analysis.

[0126] Plotting the high antigen data (which comparatively estimate specific antibody concentrations; *see* above for

details) versus the limited antigen OD illustrated that the relatively higher affinity antibodies, *e.g.*, those that bound had higher OD in the limited antigen assay while having lower amounts of IgG HER-3 antibody in the supernatant. Hybridomas from cohorts #3 and #4 for the 33 best performing antibodies in these sets of assays were advanced to cloning by limiting dilution hybridoma plating.

[0127] Alternatively, FACS analysis of HER-3 expression of Ratl/pLXSN and Ratl/HER-3 cells showed similar results (no crossreactivity with endogenous rat epitopes. In detail, $1x10^5$ cells were harvested with 10 mM EDTA in PBS, washed once with FACS buffer (PBS, 3 % FCS, 0.4 % azide) and seeded on a 96-well round bottom plate. The cells were spun for 3 minutes at 1000 rpm to remove supernatant and then resuspended with the specific HER-family antibodies (3 μg/ml). Cell suspensions were incubated on ice for 45 minutes, washed twice with FACS buffer and resuspended with secondary antibody (100 μl/well) donkey-anti-human-PE (Jackson Immunoresearch, PA) diluted 1:50 in FACS buffer. The cell suspensions were incubated on ice and in the dark for 30 minutes, washed twice with FACS buffer and analyzed (FACS, Beckman Coulter).

EXAMPLE 8: Structural Analysis of Anti-HER-3 Antibodies

[0128] The following discussion provides structural information related to antibodies prepared as described herein. In order to analyze structures of the antibodies, genes encoding the heavy and light chain fragments were amplified out of the particular hybridoma. Sequencing was accomplished as follows:

[0129] The V_H and V_L transcripts were amplified from individual hybridoma clones in 96 well plate using reverse transcriptase polymerase chain reaction (RT-PCR). Poly(A)+-mRNA was isolated from approximately $2x10^5$ hybridoma cells using a Fast-Track kit (Invitrogen). Four PCR reactions were run for each Hybridoma: two for light chain (kappa (κ), and two for gamma heavy chain (γ). The QIAGEN OneStep room temperature-PCR kit was used for amplification (Qiagen, Catalog No.210212). In the coupled room temperature-PCR reactions, cDNAs were synthesized with blend of room temperature enzymes (Omniscript and Sensiscript) using antisense sequence specific primer corresponded to C-κ, or to a consensus of the CH1 regions of Cγ genes. Reverse transcription was performed at 50 °C for 1 hr followed by PCR amplification of the cDNA by HotStarTaq DNA Polymerase for high specificity and sensitivity. Each PCR reaction used a mixture of 5'-sense primers; primer sequences were based on the leader sequences of V_H and V_K available at the Vbase website (http://vbase.mrc-cpe.cam.ac.uk/).

[0130] PCR reactions were run at 94°C for 15 min, initial hot start followed by 40 cycles of 94°C for 30 sec (denaturation), 60°C for 30 sec (annealing) and 72°C for 1 min (elongation).

[0131] PCR products were purified and directly sequenced using forward and reverse PCR primers using the ABI PRISM BigDye terminator cycle sequencing ready reaction Kit (Perkin Elmer). Both strands were sequenced using Prism dye-terminator sequencing kits and an ABI 377 sequencing machine.

[0132] Sequence analysis: Analyses of human V heavy and V kappa cDNA sequences of the HER-3 antibodies were accomplished by aligning the HER-3 sequences with human germline V heavy and V kappa sequences using Abgenix in-house software (5AS). The software identified the usage of the V gene, the D gene and the J gene as well as nucleotide insertions at the recombination junctions and somatic mutations. Amino acid sequences were also generated *in silico* to identify somatic mutations. Similar results could be obtained with commercially available sequence analysis software and publicly available information on the sequence of human V, D, and J genes, *e.g.*, Vbase (http://vbase.mrc-cpe.cam.ac.uk/).

[0133] Molecular cloning of mAb U1-59: Total RNA was extracted from the tissue culture well containing multiple hybridomas lineages, including the hybridoma lineage secreting antibody U1-59. A heavy chain variable region was amplified using 5'-leader VH family specific primers, with 3'-C-gamma primer. A major band was amplified using a VH4 primer, no other bands were visible. The VH4-34 gamma fragment was cloned into pCDNA expression vector in frame with a human gamma 1 constant region gene.

[0134] An IgM heavy chain variable region was amplified using 5' VH family specific primers with 3' mu constant region primer. A major band was amplified using VH2 primer, no other bands were visible. The VH2-5 mu fragment was cloned into pCDNA expression vector in frame with a human mu constant region gene. V kappa chains were amplified and sequenced. Four kappa chain RT-PCR products were identified. The products were sequenced and after sequence analysis via in silico translation, only three of them had open-reading frames. These three functional kappa chains were cloned out of the oligoclonal U1-59 hybridoma well identified based on V kappa gene usage as (1) VK1 A3-JK2, (2) VK1 A20-JK3 and (3) B3-JK1. All V-kappa were cloned into pCDNA expression vector in frame with a human kappa light chain constant region gene.

[0135] Transfections: Each heavy chain was transfected with each of the kappa chains in transient transfections for a total of 6 heavy chain/kappa light chain pairs. The transfection of the gamma chain with the A20 kappa chain gave poor antibody expression, while no antibody was secreted or detected when the A20 kappa chain was co-transfected with the mu chain. A total of three IgG sups and two IgM sups were available for HER-3 binding assay.

Chain	VH	D	J	Constant	ORF
Heavy	VH4-34	D1-20	JH2	Gamma	Yes
Heavy	VH2-5	D6-6	JH4b	Mu	Yes
Light	A3		JK2	Kappa	Yes
Light	A20		JK3	Kappa	Yes
Light	B3		JK1	Kappa	Yes
Light	A27		JK3	Kappa	NO

[0136] Binding activity to HER-3+ cell lines was detected in FACS with the IgG1 mAb consisting of the VH4-34 and the B3 kappa chain. No other VH/Vk combinations gave fluorescence signal above background in FACS using HER-3+ cell lines.

[0137] Binding competition of the anti-HER-3 antibodies: Multiplexed competitive antibody binning was performed as published in Jia et al. (2004) J Immunol Methods. 288, 91-98 to assess clusters of HER-3 antibodies that competed for binding to HER-3. Tested HER-3 antibodies from cohort 1 clustered into 5 bins based on competition for binding.

Bin#1	**Bin#2**	**Bin#3**	**Bin#4**	**Bin#5**
U1-42	U1-48	U1-52	U1-38	U1-45
U1-44	U1-50		U1-39	U1-40
U1-62	U1-51			U1-41
U1-46				U1-43
U1-47	U1-49			U1-61
U1-58				U1-53
				U1-55

[0138] Epitope characterization of anti-HER-3 antibodies: The epitopes of human anti-HER-3 antibodies were characterized. First a dot blot analysis of the reduced, denatured HER-3-His tagged purified ECD protein showed absence of binding by the anti-HER-3 antibodies tested (U1-59, U1-61, U1-41, U1-46, U1-53, U1-43, U1-44, U1-47, U1-52, U1-40, U1-49)) demonstrating that all had epitopes sensitive to reduction of disulfide bonds, suggesting that all had discontinuous epitopes. Next, the antibodies were mapped to defined domains in the HER-3 molecule by engineering various human-rat HER-3 chimeric molecules, based on the division of the HER-3 extra-cellular domain into four domains:

1) L1 (D1): the minor ligand-binding domain,
2) S1 (D2): the first cysteine-rich domain,
3) L2 (D3): the major ligand-binding domain, and
4) S2 (D4): the sec cysteine-rich domain.

[0139] The extra-cellular domain (ECD) of Human HER-3 cDNA was amplified from RAT1-HER-3 cells. The rat HER-3 cDNAs was amplified by RT-PCR from rat liver RNA and confirmed by sequencing. The cDNAs expressing the ECD of human and rat HER-3 were cloned into mammalian expression vectors as V5-His fusion proteins. Domains from the human HER-3 ECD were swapped into the scaffold provided by the rat HER-3 ECD by using the Mfe1, BstX1 and DraIII internal restriction sites. By this means, various chimeric rat/human HER-3 ECD HIS fusion proteins (amino acids 1-160, 161-358, 359-575, 1-358, 359-604) were constructed and expressed via transient transfection of HEK 293T cells. Expression of the constructs was confirmed using a rat polyclonal antibody against human HER-3. The human monoclonal antibodies were tested in ELISA for binding to the secreted chimeric ECDs.

[0140] Two of the human antibodies, including antibody U1-59, cross-reacted with rat HER-3. To assign binding domains, these mAbs were tested against a truncated form of HER-3 consisting of L1-S1-V5his tagged protein purified from the supernatant of HEK 293T cells transfected with a plasmid DNA encoding the expression of the L1-S1 extracellular domains of HER-3. mAb U1-59 bound to the L1-S1 protein in ELISA, implying that its epitope is in L1-S1. mAb 2.5.1 did not bind to the L1-S1 protein, implying that its epitope is in L2-S2. Further mapping of antibody U1-59 was

accomplished using SELDI time of flight mass spectroscopy with on-chip proteolytic digests of mAb-HER-3 ECD complexes.

[0141] Mapping U1-59 epitopes using SELDI: Further mapping of antibody U1-59 was accomplished using a SELDI time of flight mass spectroscopy with on-chip proteolytic digests of mAb-HER-3 ECD complexes. Protein A was covalently bound to a PS20 protein chip array and used to capture mAb U1-59. Then the complex of the PS20 protein chip and the monoclonal antibody was incubated with HER-3-His purified antigen. Next the antibody-antigen complex was digested with high concentration of Asp-N. The chip was washed, resulting in retention of only the HER-3 peptide bound to the antibody on the chip. The epitope was determined by SELDI and identified by mass of the fragment. The identified 6814 D fragment corresponds to two possible expected peptides generated from a partial digest of the HER-3-his ECD. Both overlapping peptides map to the domain S1. By coupling SELDI results with binding to a HER-3 deletion construct, the epitope was mapped to residues 251 to 325.

[0142] The location of the binding domains in the extracellular part of HER-3 that are recognized by the human anti-HER-3 mAbs are summarized in TABLE 4. The epitope domain mapping results were consistent with results from antibody competition binding competition bins, with antibodies that cross-competed each other for binding to HER-3 also mapping to the same domains on HER-3.

TABLE 4

A summary of mAb binding domains based on ELISA assay results

MAb	Rat XR	Binding domain	mAb	Rat XR	Binding domain
U1-59	Yes	S1	U1-2	No	L2
U1-61	No	L2	U1-7	No	L2
U1-41	No	L2	U1-9	No	L2
U1-46	No	S1	U1-10	No	L2
U1-53	No	L2	U1-12	No	L2
U1-43	No	L2	U1-13	No	L2
U1-44	No	S1	U1-14	No	L2
U1-47	No	S1	U1-15	No	L2
U1-52	Yes	L2S2	U1-19	No	L2
U1-40	No	L2	U1-20	No	L2
U1-49	No	L1	U1-21	No	L2
U1-21	No	L2	U1-28	No	L2
U1-22	No	L2	(U1-31)	No	L2
U1-23	No	L2	U1-32	No	L2
U1-24	No	L2	(U1-35)	No	L2
U1-25	No	L2	U1-36	No	L2
U1-26	No	L2	(U1-37)	No	L2
U1-27	No	L2			

XR = cross-reactive

EXAMPLE 9: Determination of Canonical Classes of Antibodies

[0143] Antibody structure has been described in terms of "canonical classes" for the hypervariable regions of each immunoglobulin chain (Chothia et al. (1987) J. Mol. Biol. 196:901-17). The atomic structures of the Fab and VL fragments of a variety of immunoglobulins were analyzed to determine the relationship between their amino acid sequences and the three-dimensional structures of their antigen binding sites. Chothia, *et al.* found that there were relatively few residues that, through their packing, hydrogen bonding or the ability to assume unusual phi, psi or omega conformations, were primarily responsible for the main-chain conformations of the hypervariable regions. These residues were found to occur at sites within the hypervariable regions and in the conserved β-sheet framework. By examining sequences of immu-

noglobulins having unknown structure, Chothia, *et al.* show that many immunoglobulins have hypervariable regions that are similar in size to one of the known structures and additionally contained identical residues at the sites responsible for the observed conformation.

[0144] Their discovery implied that these hypervariable regions have conformations close to those in the known structures. For five of the hypervariable regions, the repertoire of conformations appeared to be limited to a relatively small number of discrete structural classes. These commonly occurring main-chain conformations of the hypervariable regions were termed "canonical structures." Further work by Chothia et al. (Nature (1989) 342:877-83) and others (Martin et al. (1996) J. Mol. Biol. 263:800-15) confirmed that there is a small repertoire of main-chain conformations for at least five of the six hypervariable regions of antibodies.

[0145] The CDRs of each antibody described above were analyzed to determine their canonical class. As is known, canonical classes have only been assigned for CDR1 and CDR2 of the antibody heavy chain, along with CDR1, CDR2 and CDR3 of the antibody light chain. The tables below summarize the results of the analysis. The canonical class data is in the form of HCDR1-HCDR2-LCDR1-LCDR2-LCDR3, wherein "HCDR" refers to the heavy chain CDR and "LCDR" refers to the light chain CDR. Thus, for example, a canonical class of 1-3-2-1-5 refers to an antibody that has a HCDR1 that falls into canonical class 1, a HCDR2 that falls into canonical class 3, a LCDR1 that falls into canonical class 2, a LCDR2 that falls into canonical class 1, and a LCDR3 that falls into canonical class 5.

[0146] Assignments were made to a particular canonical class where there was 70 % or greater identity of the amino acids in the antibody with the amino acids defined for each canonical class. The amino acids defined for each antibody can be found, for example, in the articles by Chothia, *et al.* referred to above. TABLE 5 and TABLE 6 report the canonical class data for each of the HER-3 antibodies. Where there was less than 70 % identity, the canonical class assignment is marked with an asterisk ("*") to indicate that the best estimate of the proper canonical class was made, based on the length of each CDR and the totality of the data. Where there was no matching canonical class with the same CDR length, the canonical class assignment is marked with a letter s and a number, such as "s18", meaning the CDR is of size 18. Where there was no sequence data available for one of the heavy or light chains, the canonical class is marked with "Z".

TABLE 5

Antibody (sorted)	H1-H2-L1-L2-L3	H3length	Antibody (sorted)	H1-H2-L1-L2-L3	H3length
U1-38	3-1-4-1-1	9	U1-7	3-1-2-1-1	12
U1-39	1-1-4-1*-1	6	U1-9	3-1-2-1-1	12
U1-40	3-1-4-1-1	15	U1-10	3-1-2-1-1	12
U1-41	3-1-2-1-1	15	U1-12	3-1-2-1-1	12
U1-42	1-2-2-1-1	9	U1-13	3-1-4-1-1	7
U1-43	3-1-2-1-1	17	U1-14	3-1-2-1-1	12
U1-44	1-2-2-1-1	9	U1-15	3-1-8-1-1	14
U1-45	1-2*-2-1-1	16	U1-19	3-1-Z-Z-Z	12
U1-46	3-s18-Z-Z-Z	17	U1-20	3-1-2-1-1	19
U1-47	3-s18-2-1-1	16	U1-21	3-1-2-1-1	12
U1-48	1-1-Z-Z-Z	16	U1-22	3-1-2-1-1	12
U1-49	1-3-4-1-1	17	U1-23	3-1-2-1-1	12
U1-50	3-1-2-1-1	17	U1-24	3-1-2-1-1	12
U1-51	1-1-3-1-1	19	U1-25	3-1-2-1-1	12
U1-52	3-1-8-1-1	15	U1-26	3-1-2-1-1	12
U1-53	1-3-2-1-1	10	U1-27	3-1-2-1-1	12
U1-55	3-1-4-1-1	15	U1-28	3-1-2-1-1	12
U1-57	3-1-4-1-1	15	U1-31	1-2-2-1-1	13
U1-58	1-3-2-1-1	12	U1-32	3-1-2-1-1	12
U1-59	1-1-3-1-1	9	U1-35	1-3-2-1-1	14
U1-61.1	3-1*-2-1-1	16	U1-36	3-1-2-1-1	12

(continued)

Antibody (sorted)	H1-H2-L1-L2-L3	H3length	Antibody (sorted)	H1-H2-L1-L2-L3	H3length
U1-62	1-2-8-1-1	12	U1-37	1-2-Z-Z-Z	13
U1-2	3-1-2-1-1	12			

[0147] TABLE 6 is an analysis of the number of antibodies per class. The number of antibodies having the particular canonical class designated in the left column is shown in the right column. The four mAbs lacking one chain sequence data and thus having "Z" in the canonical assignment are not included in this counting.

[0148] The most commonly seen structure is 3-1-2-1-1: Twenty-one out of forty-one mAbs having both heavy and light chain sequences had this combination.

TABLE 6

H1-H2-L1-L2-L3	Count
1-1-3-1-1	2
1-1-4-1*-1	1
1-2-2-1-1	4
1-2-8-1-1	1
1-3-2-1-1	3
1-3-4-1-1	1
3-1-2-1-1	21
3-1-4-1-1	5
3-1-8-1-1	2
3-s18-2-1-1	1

EXAMPLE 10: Determination of Antibody Affinity

[0149] Affinity measurements of anti-HER-3 antibodies were performed by indirect FACS Scatchard analysis. Therefore, 10^5 cells of interest or SK-Br 3 cells were harvested with 10 mM EDTA in PBS, washed once with FACS buffer (PBS, 3 % FCS, 0.4 % azide) and seeded on a 96-well round bottom plate. The cells were spun for 3 min at 1000 rpm to remove supernatant and then resuspended with α-HER-3 antibody (3 μg/ml) or with antibody dilutions (100 μl/well) starting with 20 μg/ml human monoclonal antibody in FACS buffer, diluted in 1:2 dilution steps. Cell suspensions were incubated on ice for 1 hr, washed twice with FACS buffer and resuspended with secondary antibody (100 μl/well) donkey-antihuman-PE (Jackson) diluted 1:50 in FACS buffer. The cell suspensions were incubated on ice and in the dark for 30 min, washed twice with FACS buffer and analyzed (FACS, Beckman Coulter). According to the FACS Scatchard analysis, the fluorescence mean was calculated for each measurement. Background staining (= without 1st antibody) was subtracted from each fluorescence mean. Scatchard plot with x-value = fluorescence mean and y-value = fluorescence mean/concentration of mAb (nM) was generated. The KD was taken as the absolute value of 1/m of linear equation. Affinity measurements for certain antibodies selected in this manner are provided in TABLE 7.

TABLE 7

Clone	KD (nm)
U1-38	n.d.
U1-39	102
U1-40	6.7
U1-41	0.18
U1-42	n.d.
U1-43	0.57

(continued)

Clone	KD (nm)
U1-44	4
U1-52	16.8
U1-61	0.13
U1-62	20.4
U1-46	13.8
U1-47	9.38
U1-49	1
U1-50	39.3
U1-51	131.6
U1-53	0.082
U1-55.1	3.7
U1-58	6.4
U1-59	3.69
U1-24	0.06
U1-7	0.02

EXAMPLE 11: Anti-HER-3 Antibodies Induce HER-3 Receptor Endocytosis

[0150] HER-3 has been identified as a factor that can influence initiation and progression of hyperproliferative diseases through serving as an important gatekeeper of HER family mediated cell signaling. Thus, if HER-3 is effectively cleared from the cell surface/membrane by receptor internalization, cell signaling and therefore transformation and/or maintenance of cells in malignancy can be ultimately diminished or suppressed.

[0151] In order to investigate whether anti-HER-3 antibodies are capable of inducing accelerated endocytosis of HER-3, the relative amount of HER-3 molecules on the cell surface after 0.5 and 4 hr incubation of the cells with anti-HER-3 antibodies were compared. $3x10^5$ cells were seeded in normal growth medium in 24-well dish and left to grow overnight. Cells were preincubated with 10 μg/ml anti-HER-3 mAbs in normal growth medium for the indicated times at 37°C. Cells were detached with 10 mM EDTA and incubated with 10 μg/ml anti-HER-3 mAbs in wash buffer (PBS, 3 % FCS, 0.04 % azide) for 45 min at 4°C. Cells were washed twice with wash buffer, incubated with donkey-antihuman-PE secondary antibody (Jackson) diluted 1:100 for 45 min at 4°C, washed twice with wash buffer and analyzed by FACS (Beckman-Coulter, EXPO). Percent internalization was calculated based on the reduction of the mean fluorescence intensity of anti-HER-3 treated samples relative to control-treated samples. These experiments demonstrated that treatment of cells with anti-HER-3 antibodies led to internalization of the receptor. *See,* Figure 5 of US Publication No. 20080124345.

EXAMPLE 12: Inhibition of Ligand Binding to Human Cancer Cells SKBr3 by Human Anti-HER-3 Antibodies

[0152] Radioligand competition experiments were performed in order to quantitate the ability of the anti-HER-3 antibodies to inhibit ligand binding to HER-3 in a cell based assay. Therefore, the HER-3 receptor binding assay was performed with $4x10^5$ SK-BR-3 cells which were incubated with varying concentrations of antibodies for 30 min on ice. 1.25 nM [I^{125}]-α-HRG/[^{125}I]-β-HRG were added to each well and the incubation was continued for 2 hr on ice. The plates were washed five times, air-dried and counted in a scintillation counter. The antibodies were capable of specifically reducing the binding of [^{125}I]-α-HRG/[^{125}I]-β-HRG to cells expressing endogenous HER-3. *See*, Figures 6a-6e of US Publication No. 20080124345.

EXAMPLE 13: Inhibition of Ligand-induced HER-3 Phosphorylation by Human Anti-HER-3 Antibodies

[0153] ELISA experiments were performed in order to investigate whether the antibodies are able to block ligand β-HRG-mediated activation of HER-3. Ligand-mediated HER-3 activation was detected by increased receptor tyrosine phosphorylation.

Day 1: 1 x 96 well dish was coated with 20 μg/ml Collagen I in 0,1 M acetic acid for 4 hr at 37°C. $2.5x10^5$ cells were seeded in normal growth medium
Day 2: Cells were starved in 100 μl serum free medium for 24 hr.
Day 3: Cells were preincubated with 10 μg/ml anti-HER-3 mAbs for 1 hr at 37°C and then treated with 30 ng/ml β-HRG-EGF domain (R&D Systems) for 10 min. Medium was flicked out and cells were fixed with 4 % formaldehyde solution in PBS for 1 hr at room temperature. Formaldehyde solution was removed and cells were washed with wash buffer (PBS/0.1 % Tween 20). Cells were quenched with 1 % H_2O_2, 0.1 % NaN_3 in wash buffer and incubated for 20 min at room temperature, then blocked with NET-Gelantine for 5 hr at 4°C. Primary antibody phospho-HER-3 (Tyr1289) (polyclonal rabbit; Cell signaling #4791; 1:300) was added overnight at 4°C.
Day 4: The plate was washed 3x with wash buffer, then incubated with anti-rabbit-POD diluted 1:3000 in PBS - 0.5 % BSA was added to each well and incubated for 1.5 hr at room temperature. The plate was washed 3x with wash buffer and once with PBS. Tetramethylbenzidine (TMB, Calbiochem) was added and monitored at 650 nm. The reaction was stopped by addition of 100 μl 250 nM HCl and the absorbance was read at 450 nm with a reference wavelength of 650 nm using a Vmax plate reader (Thermo Lab Systems).

[0154] These experiments demonstrated that anti-HER-3 antibodies were able to reduce ligand-mediated HER-3 activation as indicated by decreased receptor tyrosine phosphorylation. *See,* Figure 7a of US Publication No. 20080124345.

[0155] To test potency of mAb U1-53 to inhibit ligand induced HER-3 activation, MCF-7 cells were starved for 24 hr, incubated with mAb U1-53 for 1 hr at 37°C and stimulated with 10 nM HRG-β for 10 min. Lysates were transferred to 1B4 (mouse anti-HER-3 mAb) ELISA plates and phosphorylation of HER-3 was analyzed with antibody 4G10. Phosphorylation of HER-3 was almost completely inhibited in a dose dependent manner with an IC_{50} of 0.14 nM. *See*, Figure 7b of US Publication No. 20080124345.

EXAMPLE 14: Inhibition of Ligand-induced p42/p44 MAP-Kinase Phosphorylation by Human Anti-HER-3 Antibodies

[0156] Next ELISA experiments were performed in order to investigate whether the antibodies are able to block ligand β-HRG-mediated activation of p42/p44 MAP-Kinase. Ligand-mediated HER-3 activation was detected by increased protein (Thr202/Tyr204) phosphorylation.

Day 1: 1 x 96 well dish was coated with 20 μg/ml Collagen I in 0,1 M acetic acid for 4 hr at 37°C. $3x10^5$ cells were seeded in normal growth medium
Day 2: Cells were starved in 100 μl serum free medium for 24 hr.
Day 3: Cells were preincubated with 5 μg/ml anti-HER-3 mAbs for 1 hr at 37°C and then treated with 20 ng/ml β-HRG-EGF domain (R&D Systems) for 10 min. Medium was flicked out and cells were fixed with 4 % formaldehyde solution in PBS for 1 hr at room temperature. Formaldehyde solution was removed and cells were washed with wash buffer (PBS/0.1 % Tween 20). Cells were quenched with 1 % H_2O_2, 0.1 % NaN_3 in wash buffer and incubated for 20 min at room temperature, then blocked with PBS/0.5 % BSA for 5 hr at 4 °C. Primary antibody phospho-p44/p42 MAP Kinase (Thr202/Tyr204) (polyclonal rabbit; Cell signaling #9101; 1:3000) was added overnight at 4°C.
Day 5: The plate was washed 3x with wash buffer, then incubated with anti-rabbit-HRP diluted 1:5000 in PBS - 0.5 % BSA was added to each well and incubated for 1.5 hr at room temperature. The plate was washed 3x with wash buffer and once with PBS. Tetramethylbenzidine (TMB, Calbiochem) was added and monitored at 650 nm. The reaction was stopped by addition of 100 μl 250 nM HCl and the absorbance was read at 450 nm with a reference wavelength of 650 nm using a Vmax plate reader (Thermo Lab Systems). These experiments revealed that the antibodies were able to reduce ligand-mediated p42/p44 MAP-Kinase activation as indicated by decreased phosphorylation. *See,* Figure 8 of US Publication No. 20080124345.

EXAMPLE 15: Inhibition of β-HRG-induced Phospho-AKT Phosphorylation by Human Anti-HER-3 Antibodies

[0157] In the following ELISA experiment we investigated whether the anti-HER-3 antibodies are able to block ligand β-HRG-mediated activation of AKT-Kinase. Ligand-mediated AKT activation was detected by increased protein (Ser473) phosphorylation.

Day 1: 1 x 96 well dish was coated with 20 μg/ml Collagen I in 0,1 M acetic acid for 4 hr at 37°C. $3x10^5$ cells were seeded in normal growth medium
Day 2: Cells were starved in 100 μl serum free medium for 24 hr.
Day 3: Cells were preincubated with 5 μg/ml anti-HER-3 mAbs for 1 hr at 37°C and then treated with 20 ng/ml β-HRG-EGF domain (R&D Systems) for 10 min. Medium was flicked out and cells were fixed with 4 % formaldehyde

solution in PBS for 1 hr at room temperature. Formaldehyde solution was removed and cells were washed with wash buffer (PBS/0.1 % Tween 20). Cells were quenched with 1 % H_2O_2, 0.1 % NaN_3 in wash buffer and incubated for 20 min at room temperature, then blocked with PBS/0.5 % BSA for 5 hr at 4 °C. Primary antibody phospho-Akt (Ser473) (polyclonal rabbit; Cell signaling #9217; 1:1000) was added overnight at 4°C.

Day 4: The plate was washed 3x with wash buffer, then incubated with anti-rabbit-HRP diluted 1:5000 in PBS-0.5 % BSA was added to each well and incubated for 1.5 hr at room temperature. The plate was washed 3x with wash buffer and once with PBS. Tetramethylbenzidine (TMB, Calbiochem) was added and monitored at 650 nm. The reaction was stopped by addition of 100 μl 250 nM HCl and the absorbance was read at 450 nm with a reference wavelength of 650 nm using a Vmax plate reader (Thermo Lab Systems). The anti-HER-3 antibodies were able to reduce β-HRG-mediated AKT as indicated by decreased phosphorylation. *See,* Figure 9 of US Publication No. 20080124345.

EXAMPLE 16: Inhibition of a-HRG/β-HRG-mediated MCF7 Cell proliferation by Human Anti-HER-3 Antibodies

[0158] *In vitro* experiments were conducted in order to determine the ability of the antibodies to inhibit HRG-stimulated cell proliferation. 2000 MCF7 cells were seeded in FCS-containing medium on 96-well plates overnight. Cells were preincubated in quadruplicates with antibody diluted in medium with 0.5 % FCS for 1 hr at 37°C. Cells were stimulated with 30 ng/ml α- or 20 ng/ml β-HRG (R&D Systems) by adding ligand directly to antibody solution and were then left to grow for 72 hr. ALAMAREBLUE™ (BIOSOURCE) was added and incubated at 37°C in the dark. Absorbance was measured at 590 nm every 30 min. The data were taken 90 min after addition of alamar blue. These studies showed that representative antibodies could inhibit HRG-induced cell growth in human cancer cells. *See,* Figure 10 of US Publication No. 20080124345.

EXAMPLE 17: Inhibition of β-HRG-induced MCF7 Cell Migration by Human Anti-HER-3 Antibodies

[0159] Transmigration experiments were performed in order to investigate whether the antibodies block cell migration. Serum-starved MCF7 cells were preincubated by adding the indicated amount of antibody to the cell suspension and incubating both for 45 min at 37°C. 500 μl cell suspension (50,000 cells) was then placed in the top chamber of collagen I-coated transwells (BD Falcon, 8 μm pores). 750 μl medium (MEM, amino acids, Napyruvate, Pen.-Strept., 0,1 % BSA, without fetal calf serum) alone or containing the ligands β-HRG-EGF domain (R&D Systems) were used in the bottom chamber. Cells were left to migrate for 8 hr at 37°C and were stained with DAPI. Stained nuclei were counted manually; percent inhibition was expressed as inhibition relative to a control antibody. These experiments demonstrated that representative anti-HER-3 antibodies could reduce HRG-induced cell migration. *See*, Figure 11 of US Publication No. 20080124345.

EXAMPLE 18: Colony Formation Assay (Soft Agar Assay)

[0160] Soft agar assays were conducted in order to investigate the ability of the anti-HER-3 antibodies to inhibit anchorage independent cell growth. The soft agar colony formation assay is a standard *in vitro* assay to test for transformed cells, as only such transformed cells can grow in soft agar.

[0161] 750 to 2000 cells (depending on the cell line) were preincubated with indicated antibodies at 10 μg/ml in IMDM medium (Gibco) for 30 min and resuspended in 0.4 % Difco noble agar. The cell suspension was plated on 0.75 % agarose underlayer containing 20 % FCS in quadruplicate in a 96-well plate. Colonies were allowed to form for 14 days, and were then stained with 50 μl MTT (0.5 mg/ml in PBS) overnight, and counted with a Scanalyzer HTS camera system (Lemnatec, Wuerselen). Anti-HER-3 antibodies were able to reduce anchorage independent cell growth of MDA-MB361 and NCI-ADR breast cancer cells, MKN-28 gastric cancer cells, HT144 melanoma cells, Skov3 ovary carcinoma cells, PPC-1 prostate cancer cells, BX-PC3 pancreas cancer cells, A431 epidermoid carcinoma cells, and lung carcinoma cells. *See,* Figures 12a-12i of US Publication No. 20080124345.

EXAMPLE 19: Human Anti-HER-3 Antibodies Inhibit Human Breast Carcinoma Growth in Nude Mice

[0162] The anti-tumor efficacy of therapeutic antibodies is often evaluated in human xenograft tumor studies. In these studies, human tumors grow as xenografts in immunocompromised mice and therapeutic efficacy is measured by the degree of tumor growth inhibition. In order to determine, if the anti-HER-3 antibodies interfere with tumor growth of human breast cancer cells in nude mice, $5x10^6$ T47D cells were implanted in female NMRI nude/nude mice. Tumors were subcutaneous, grown on the back of the animal. Treatments began when tumors reached a mean volume of 20 mm^3; eight days post implantation. Prior to first treatment, mice were randomized and statistical tests performed to assure uniformity in starting tumor volumes (mean, median and standard deviation) across treatment groups. Treatment

started with a loading dose of 50 mg/kg followed by 25 mg/kg injections once a week by intraperitoneal injection. A control arm received doxorubicin (pharmaceutical grade). All animals were supplemented with 0.5 mg/kg/week oestrogen injected i.p. Details of the treatment groups are given in TABLE 8 below. These studies demonstrated that administration of an anti-HER-3 antibody resulted in reduction of tumor growth. *See*, Figure 13 of US Publication No. 20080124345.

TABLE 8

Gr.	N	1st Compound	Loading (mg/kg)	Weekly dose (mg/kg)	Route	Schedule
1.	10	PBS		--	i.p.	once/week
2.	10	Doxorubicin		8mg/kg	i.v.	once/week*
3.	10	U1-53	50mg/kg 20ml/kg	25mg/kg 10ml/kg	i.p.	once/week
* doxorubin treatment as described by Boven et al., Cancer Research, 1992.						

EXAMPLE 20: Human Anti-HER-3 Antibodies Inhibit Human Pancreatic Tumor Growth in SCID Mice

[0163] To test the therapeutic potential of anti-HER-3 antibodies in other solid tumor types the anti-HER-3 antibodies, U1-53 and U1-59, were tested in mice with established tumors derived from the human pancreatic tumor cell line BxPC3. As controls sets of mice treated with either the vehicle control, PBS, or the established therapeutic antibody, Erbitux, were included. $5x10^6$ BxPC3 cells were inoculated subcutaneously without Matrigel into CB 17 SCiD mice. Mice bearing established tumors with a mean volume of $140mm^2$ received 50mg/kg of U1-53, U1-59, Erbitux or the equivalent volume of PBS via intraperitoneal injection. Thereafter the mice received once weekly 25mg/kg injections for the duration of the study.

[0164] U1-53 and U1-59 reduced the growth of the human pancreatic tumors in a cytostatic fashion. *See,* Figure 14 of US Publication No. 20080124345. Notably, in this experiment, U1-53 and U1-59 were more effective than the EGF-R-targeting antibody Erbitux at delaying tumor growth. These studies demonstrated the therapeutic efficacy of anti-HER-3 antibodies in comparison to a benchmark therapeutic agent.

EXAMPLE 21: Combining the Human Anti-HER-3 Antibodies with Anti-EGF-R Antibodies Increases Anti-tumor Activity

[0165] The monotherapy of hyperproliferative diseases with targeted antibodies is often hampered by problems such as, on the one hand, the development of resistance to drugs, and on the other hand, a change in the antigenicity. For example, loss of antigenicity after prolonged treatment may render tumor cells insensitive to therapeutic antibodies, since those tumor cells that do not express or have lost the targeted antigen have a selective growth advantage. These problems might be evaded by using the antibodies in combination with a therapeutic antibody that targets a different receptor on the tumor cells, or another antineoplastic agent. Intervening in multiple signaling pathways or even related pathways but at multiple intervention steps might also provide therapeutic benefit. These combined treatment modalities are likely to be more efficacious, because they combine two anti-cancer agents, each operating via a different mechanism of action.

[0166] In order to demonstrate the feasibility of the anti-HER-3 antibodies U1-53 and U1-59 as suitable combination agents, we compared monotherapeutic administrations of U1-53 or U1-59 with those in which either U1-53 or U1-59 was combined with the anti-EGR specific antibody, Erbitux. $5x10^6$ BxPC3 cells were inoculated subcutaneously with Matrigel into CB17 SCID mice. After tumor volumes had reached 200 mm^3, mice were randomized into individual treatment groups. Weekly intraperitoneal administrations of U1-53, U1-59 and Erbitux as single agents or combinations of either anti-HER-3 antibodies with Erbitux or as a cocktail of two anti HER-3 antibodies were performed. All antibodies were dosed at a single loading dose of 50 mg/kg/week, followed by weekly injections of 25 mg/kg for six weeks. Control arms received bi-weekly administrations of Gemcitabine (120 mg/kg), weekly pooled human IgG or weekly vehicle (PBS) injections. The regimens are detailed in TABLE 9 below.

TABLE 9

Gr.	N	Compound	Loading dose (mg/kg)	Weekly dose (mg/kg)	Route	Schedule
1	12	PBS	20ml/kg	10ml/kg	q7d	i.p.
2	12	Pooled human IgG	50mg/kg	25mg/kg	q7d	i.p.
3	12	U1-53	50mg/kg	25mg/kg	q7d	i.p.

(continued)

Gr.	N	Compound	Loading dose (mg/kg)	Weekly dose (mg/kg)	Route	Schedule
4	12	U1-59	50mg/kg	25mg/kg	q7d	i.p.
5	12	Erbitux	50mg/kg	25mg/kg	q7d	i.p.
6	12	U1-53 + Erbitux	25mg/kg each	12.5mg/kg each	q7d	i.p.
7	12	U1-59 + Erbitux	25mg/kg each	12.5mg/kg each	q7d	i.p.
8	12	U1-53 + U1-59	25mg/kg each	12.5mg/kg each	q7d	i.p.
9	12	Gemcitabine	none	120 mg/kg	2x weekly	i.p.

[0167] Antibodies U1-53 and U1-59, when administered as single agents, delayed the growth of the human pancreatic tumors to the same degree as Gemcitabine, which is often used as a standard anti-pancreatic cancer chemotherapy. Co-administration of Erbitux with U1-53 or U1-59 resulted in a significantly greater reduction of tumor growth than observed with either single agent administration of U1-53, U1-59 or Erbitux. Thus, a beneficial therapeutic response can be achieved by combining the anti-HER-3 antibodies with suitable antibodies that target separate tumor antigens. *See*, Figure 15 of US Publication No. 20080124345.

[0168] In summary, the anti-HER-3 antibodies had potent therapeutic efficacy against human tumors *in vivo.* They can be effectively combined with other anti-neoplastic therapeutics for increased anti-tumor activity.

EXAMPLE 22: Human Anti-HER-3 Antibodies Inhibit Human Melanoma Tumor Growth in nu/nu Mice

[0169] Members of the erbB family of receptors, including HER-3, are abnormally expressed in a large variety of epithelial cancers and they are known to play important roles in the growth and survival of many these solid tumors. These tumors include melanomas, head and neck squamous cell cancers, non-small cell lung cancers and prostate, glioma, gastric, breast, colorectal, pancreatic, ovarian cancers. In order to verify, that the anti-HER-3 antibodies are not restricted in their anti-cancer activity to individual tumor types, *e.g.,* pancreatic cancers (*see,* Example 21), but can be used as therapeutics against many HER-3-dependent tumors, we tested U1-53 and U1-59 in additional xenograft studies. Human melanoma cells (5×10^5), HT144, were injected subcutaneously into CB17 SCID mice, followed by immediate subsequent intraperitoneal injection of 50mg/kg of U1-53 and U1-59, the equivalent volume of PBS or Dacarbacin (DITC) at 200mg/kg. Thereafter, mice received 25mg/kg of U1-53 or U1-59 once weekly, whereas DITC was given once every two weeks at 200mg/kg.

[0170] The median tumor volumes from each treatment group were calculated. Administration of the antibodies resulted in growth reduction of the human melanomas when compared to tumors that had been treated with the vehicle control. *See,* Figure 16 of US Publication No. 20080124345. These results demonstrate that the antibodies are not restricted in their therapeutic potential and target a wide variety of HER-3 expressing cancers.

EXAMPLE 23: Human Anti-HER-3 Antibodies Inhibit Growth of Colon Carcinoma Xenografts in Mice

[0171] HT-29 human colon carcinoma cells were suspended in medium with 2:1 ratio of Matrigel to a final concentration of 10×10^6 cells/ml. 0.2 ml of cell suspension were injected s.c. into the right flank of 4-5-week-old CD1 nu/nu mice. A total of 95 mice were used.

[0172] The mice were randomly assigned to control and treatment groups. The treatment started on the same day. Duration of treatment was 29 days. Upon completion of the study, three tumors per group were collected 3 hours after administration of treatment. The tumours were fast-frozen and kept at -80°C.

[0173] The following treatment protocol was carried out:

- Control group: non-specific human IgG 25 mg/kg, twice weekly, intraperitoneal
- Treatment group: antibody U1-53, 25 mg/kg, twice weekly, intraperitoneal
- Treatment group: antibody U1-7, 25 mg/kg, twice weekly, intraperitoneal
- Treatment group: antibody U1-59, 25 mg/kg, twice weekly, intraperitoneal
- Treatment group 5-FU: 5-fluorouracil, 50 mg/kg, 9d x 5, intraperitoneal

[0174] The median tumor volumes from each group were calculated. Administration of the antibodies resulted in growth reduction of the HT-29 colon carcinoma tumors when compared to tumors that had been treated with non-specific human IgG1. *See,* Figure 17 of US Publication No. 20080124345.

EXAMPLE 24: Human Anti-HER-3 Antibodies Inhibit Lung Cancer Growth in Mice

[0175] Calu-3 human non-small cell lung cancer cells were suspended in medium with 1:1 ratio of Matrigel to a final concentration of 5 x 10^6 cells/ml. 0.05 ml of cell suspension were injected s.c. into the right flank of 9-week-old female CB17 scid mice. A total of 60 mice were used.

[0176] The mice were randomly selected to control and treatment groups. Treatment started on the same day. The duration of treatment was 32 days.

[0177] The following treatment protocol was carried out:

PBS vehicle group

- hG control group: non-specific human IgG: 25 mg/kg, twice weekly, intraperitoneal
- Treatment group antibody U1-53, 25 mg/kg, twice weekly, intraperitoneal
- Treatment group antibody U1-7, 25 mg/kg, twice weekly, intraperitoneal
- Treatment group antibody U1-59, 25 mg/kg, twice weekly, intraperitoneal

[0178] The median tumor volumes from each control and treatment group were calculated. Administration of the antibodies resulted in growth reduction of the human non-small lung cancer xenografts when compared to tumors that had been treated with the PBS vehicle control or non-specific human IgG. *See*, Figure 18 of US Publication No. 20080124345.

EXAMPLE 25: Human Anti-HER-3 Antibodies Inhibit Human Pancreatic Tumor Growth in Balb/C-Mice

[0179] Human pancreatic BxPC3 tumor cells were suspended in medium with a 2:1 ratio of Matrigel to a final concentration of 5 x 10^6 cells per ml. 0.2 ml of cell suspension were injected s.c. into the right flank of 5-7- week-old female BalbC nu/nu mice. A total of 100 mice were used.

[0180] The mice were randomly distributed into control and treatment groups. The treatment started on the same day. The treatment duration was 27 days.

[0181] The following treatment protocol was carried out:

- hIgG control group: non-specific human IgG2, 25 mg/kg, twice weekly, intraperitoneal
- Treatment group antibody U1-53, 25 mg/kg, twice weekly, intraperitoneal
- Treatment group antibody U1-7, 25 mg/kg, twice weekly, intraperitoneal
- Treatment group antibody U1-59, 25 mg/kg, weekly, intraperitoneal
- Gemzar treatment group, gemcitabine, 80 mg/kg, weekly, intraperitoneal

[0182] The median tumor volumes from each control and treatment group were calculated. Administration of the antibodies resulted in growth reduction of the human pancreatic tumors when compared to tumors that had been treated with non-specific human IgG or with Gemzar. *See*, Figure 19 of US Publication No. 20080124345.

[0183] The inhibition of HER-3 in the human pancreatic tumors could also be shown in a pharmacodynamic experiment. The BxPC3 tumor xenografts were grown as described above. 3 mice were treated with 500 μg of an IgG1 control antibody and 3 mice were treated with 500 μg of the anti-HER-3 antibody U1-59. The mice were treated on day 1 and day 4 and then sacrificed on day 5 to measure the antibody-dependent inhibition of HER-3 phosphorylation (pHER-3).

[0184] The tumors were homogenized in a standard RIPA buffer with protease inhibitors. 50 μg clear lysate was separated on a 4-20 % Tris-glycine gel, transferred onto a nitrocellulose membrane and blocked in 3 % bovine serum albumin (BSA). Immunoblotting was performed using an anti-pHER-3 antibody (antibody 21D3, Cell Signaling technology). An anti-actin antibody (AB a-2066, Sigma) was used as a control.

[0185] Expression was detected by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ). The images were captured with the Versadoc 5000 Imaging System (BioRad, Hercules, CA). After administration of the human anti-HER-3-antibody U1-59, phosphorylation of HER-3 was no longer detectable. *See,* Figure 20 of US Publication No. 20080124345. Thus, the antibodies were capable of significantly reducing HER-3 activation in human pancreatic tumor cells.

EXAMPLE 26: U1-59 Inhibits Tumor Growth in Combination with a Second Agent in Xenograft Studies

[0186] Calu-3 NSCLC tumor xenograft models were used to evaluate the effectiveness of an anti-HER-3 antibody (U1-59), either alone or in combination with panitumamab or erlotinib. To determine *in vivo* efficacy, mice bearing ~200 mm^3 Calu-3 NSCLC xenografts were treated twice a week with anti-HER family inhibitors or control. Other experiments

were done with A549 cells. In the combination studies with panitumumab, IgG1 was used as a negative control for U1-59, and IgG2 was used as a negative control for panitumumab. As shown in Figure 1, while 100 μg of U1-59 or 100 μg of panitumumab alone greatly reduced tumor growth as compared to control, the combination of 100 μg of each of the two agents completely inhibited tumor growth ($p < 0.0001$ for the combination vs. either agent alone). In the combination studies with erlotinib, IgG1 was used as a negative control for U1-59, and erlotinib vehicle was used as a negative control for erlotinib. As shown in Figure 2, the combination of 100 μg U1-59 and 25 μg erlotinib had a greater inhibitory effect than either agent alone. The combination of UI-59 with erlotinib was significantly more effective than U1-59 alone ($p = 0.0376$).

EXAMPLE 27: U1-59 in Combination with HER Inhibitors Inhibits Anchorage-independent Growth of Breast and Ovarian Cancer Cells

[0187] Experiments were conducted to evaluate the effect of U1-59 in combination with the HER inhibitors pertuzumab, trastuzumab, or cetuximab on anchorage-independent growth of SkBr-3 (basal or HRG stimulated) and MDA-MB-435 (basal) cancer cells. IgG was used as a negative control for all studies. Tumor cell colonies formed in the absence or presence of HRG for 6 to 10 days and were stained with MTT for 4 to 6 hours and quantified. U1-59 as a single agent did not inhibit colony growth of MDA-MB 435 cells, but inhibited colony growth by 50% in the SkBr-3 cells ($p<0.001$), and up to 95% when combined with other HER inhibitors ($p<0.05$). For example, the combination of 5 μg/ml pertuzumab or trastuzumab with 5 μg/ml U1-59 reduced anchorage-independent growth in basal SkBr-3 breast cancer cells significantly more than either agent alone (Figure 3), pertuzumab, trastuzumab, or cetuximab in combination with U1-59 were significantly ($p<0.006$) more effective than U1-59 alone in HRG stimulated SkBr-3 cells (Figure 4). Similarly, combinations of U1-59 with either pertuzumab, trastuzumab or cetuximab inhibited colony formation of basal ovarian cancer cells (MDA-MB-435) significantly better ($p<0.002$) than U1-59 alone (Figure 5).

EXAMPLE 28: U1-59 in Combination with HER-2 Inhibitors or Chemotherapeutic Agents Reduces Cancer Cell Proliferation

[0188] Studies were conducted to evaluate the effect of U1-59 in combination with HER-2 inhibitors or chemotherapeutic agents on cancer cell proliferation. In particular, the following experiments were conducted in MDA-MB-175VII breast cancer cells:

U1-59 and Trastuzumab

Control = DMSO + 75 μg/ml IgG1 + PBS

10 μg/ml U1-59
75 μg/ml Trastuzumab
10 μg/ml U1-59 + 75 μg/ml Trastuzumab

U1-59 and Lapatinib

Control = DMSO + 150 μg/ml IgG1

73.5 μg/ml U1-59
0.1 μM Lapatinib
73.5 μg/ml U1-59 + 0.1 μM Lapatinib

U1-59 and Gemcitibine

Control = DMSO + 75 μg/ml IgG1 + PBS

10 μg/ml U1-59
1 μg/ml Gemcitibine
10 μg/ml U1-59 + 1 μg/ml Gemcitibine

U1-59 and Cisplatin

Control = DMSO + 75 μg/ml IgG1 + PBS

10 μg/ml U1-59
1 μg/ml Cisplatin
10 μg/ml U1-59 + 1 μg/ml Cisplatin

[0189] MDA-MB-175VII breast cancer cells were incubated with U1-59 and/or the other agents for 1 hour prior to HRG stimulation. After four days, the growth of treated cells was measured with ALOMAR BLUE™. In these assays, U1-59 reduced HRG-stimulated MDA-MB-175VII proliferation up to 40% ($p<0.05$) as a single agent, and up to 80% ($p<0.05$) when combined with trastuzumab or lapatinib (Figures 6A and 6B). Of note, additive activity also was observed in MDA-MB-175VII cells when U1-59 was combined with standard of care chemotherapeutics (gemcitabine and cisplatin; $p<0.05$ vs. either single agent alone) (Figures 6C and 6D). In each of these experiments, the combination of U1-59 with the HER-2 inhibitor was more effective at reducing proliferation of MDA-MB175VII cells than either agent alone.

[0190] Similar experiments were conducted with U1-59 and pertuzumab, trastuzumab, or lapatinib in HRG stimulated ZR-75-30 breast cancer cells and HRG stimulated BT474 breast cancer cells (Figures 7 and 8, respectively). In each case, the combination of U1-59 and lapatinib had the greatest inhibitory effect on cell proliferation. Compared to single agent treatment alone, the combination of U1-59 with pertuzumab or trastuzumab or lapatinib was significantly ($p < 0.004$) more effective than U1-59 alone. Combining U1-59 with one or more of pertuzumab, trastuzumab, and cetixumab in HRG stimulated DLD-1 colon cancer cells and HRG stimulated HCC-1569 breast cancer cells had similar effects, as shown in Figures 9 and 10. In addition, combinations of U1-59 with trastuzumab or lapatinib in HRG stimulated SkBr-3 breast cancer cells also were more effective than U1-59 alone ($p < 0.004$) (Figure 11).

[0191] In additional experiments, Head and Neck cancer cells (FaDu) were cultured in growth medium (MEM + 10% FBS + 1X PSG) and treated with IgG controls, U1-59, panitumumab or a combination of U1-59 with panitumab. After incubation for 5 days at 37°C, proliferation was measured with ALOMAR BLUE™. As a single agent, U1-59 reduced proliferation of FaDu cells by 15% to 20%, whereas the combination of U1-59 with panitumumab resulted in more than 80% reduction. The combination of U1-59 with panitumumab resulted in a significant ($p = 0.001$ vs. best single agent activity) improvement over the use of either agent alone (Figure 12).

EXAMPLE 29: U1-59 in Combination with Other HER Inhibitors Inhibits Signal Transduction

[0192] The effect of U1-59 either alone or in combination with cetuximab, pertuzumab, trastuzumab, or lapatinib on signal transduction was measured in unstimulated MDA-MB-175VII breast cancer cells, HRG stimulated SkBr-3 breast cancer cells, HRG stimulated Ls174T colon cancer cells, and HRG stimulated HCC-1569 breast cancer cells. Cells were treated with agents as indicated in Figures 13-16, and phosphorylation of HER-3, Akt, and ERK was evaluated by Western blotting with phospho-specific antibodies. The combination of U1-59 with either pertuzumab, trastuzumab, or lapatinib further reduced phosphorylation of HER-3, Akt and ERK in all cell types tested as compared to single agent treatments. The combination of U1-59 with cetuximab appeared to synergize less efficiently in these assays.

[0193] Similar studies were conducted in A549 alveolar epithelial cells (Figure 17) and Calu3 NSCLC cells (Figure 18) treated with U1-59 alone or U1-59 in combination with panitumumab or lapatinib, using Western blotting to evaluate phosphorylation of Akt, EGF-R, HER-2, HER-3, HER-4, and ERK. The combination of U1-59 with panitumumab had the greatest apparent effect on HER-3 phosphorylation in A549 cells, while the combination was more effective with regard to Akt and EGF-R phosphorylation in Calu3 cells.

[0194] Additional experiments were conducted to evaluate the *in vitro* efficacy and anchorage-independent growth of A549 cells treated with 10 μg/mL U1-59, other HER family Abs, or control mAb in serum containing medium. Tumor cell colonies formed in the absence of exogenous ligand for 10 days and were stained with MTT and quantified using a Scanalyzer HTS camera imaging system. U1-59 inhibited colony growth by 50% ($p<0.001$) in the A549 cell line and resulted in tumor stasis in the A549 NSCLC xenograft model vs. IgG control or other HER mAbs ($p<0.05$).

[0195] These results demonstrate that U1-59 inhibits proximal and distal HER-3 oncogenic signaling in breast cell lines *in vitro,* and that breast cancer cells are sensitive to U1-59 treatment as a single agent and in combination with anti-HER agents.

EXAMPLE 30: U1-59 sensitizes lapatinib for *in vivo* Activity

[0196] To evaluate the combined effects of U1-59 and lapatinib *in vivo,* mice were implanted with human breast cancer

cells (HCC-1569) and treated with U1-59 and lapatinib either alone or in combination. Tumors were allowed to reach sizes greater than or equal to 100 mm^3, and mice were subsequently treated with control, lapatinib, U1-59, or a combination of U1-59 and lapantinib. As shown in Figure 19, U1-59 alone did not inhibit HCC-1569 tumor growth, and lapatinib alone caused some, but not significant tumor growth inhibition compared to the control (p=0.16). The combination of lapatinib with U1-59, however, resulted in significant inhibition of tumor growth ($p < 0.02$ vs. control or $p < 0.05$ vs. lapatinib).

[0197] These results indicate that the combination of U1-59 and lapatinib resulted in synergistic inhibition of HCC-1569 tumor growth in *vivo.* This result is particularly interesting and encouraging as it shows that even tumor types that may not respond to U1-59 or lapatinib alone, can be very effectively treated with thhe combination of both.

EXAMPLE 31: Use of anti-HER-3 Antibodies as Diagnostic Agents

[0198] Anti-HER-3 mAb can be used in the diagnostic of malignant diseases. HER-3 is expressed on tumor cells in a very distinct way compared to normal tissue and, therefore, an expression analysis of HER-3 would assist in the primary diagnosis of solid tumors, staging and grading of solid tumors, assessment of prognostic criteria for proliferative diseases and neoplasias and risk management in patients with HER-3 positive tumors.

A. Detection of HER-3 antigen in a sample

[0199] An Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of HER-3 antigen in a sample is developed. In the assay, wells of a microtiter plate, such as a 96-well microtiter plate or a 384-well microtiter plate, are adsorbed for several hr with a first fully human monoclonal antibody directed against the HER-3 antigen. The immobilized antibody serves as a capture antibody for any of the HER-3 antigen that may be present in a test sample. The wells are rinsed and treated with a blocking agent such as milk protein or albumin to prevent nonspecific adsorption of the analyte.

[0200] Subsequently the wells are treated with a test sample suspected of containing the HER-3 antigen, or with a solution containing a standard amount of the HER-3 antigen. Such a sample is, for example, a serum sample from a subject suspected of having levels of circulating HER-3 antigen considered to be diagnostic of a pathology. After rinsing away the test sample or standard, the wells are treated with a second fully human monoclonal anti-HER-3 antibody that is labelled by conjugation with biotin. The labeled anti-HER-3 antibody serves as a detecting antibody. After rinsing away excess secondary antibody, the wells are treated with avidin-conjugated horseradish peroxidase (HRP) and a suitable chromogenic substrate. The concentration of the HER-3 antigen in the test samples is determined by comparison with a standard curve developed from the standard samples.

B. Detection of HER-3-antigen in Immunohistochemistry (IHC)

[0201] In order to determine HER-3-antigen in tissue sections by IHC, Paraffin-embedded tissues are first deparaffinized in xylene for 2 x 5 min and then hydrated with 100% Ethanol 2 x 3 min, 95% Ethanol 1 min and rinsed in distilled water. Antigenic epitopes masked by formalin-fixation and paraffin-embedding are exposed by epitope unmasking, enzymatic digestion or saponin. For epitope unmasking paraffin sections are heated in a steamer, water bath or microwave oven for 20-40 min in a epitope retrieval solution as for example 2N HCl solution (pH 1.0). In the case of an enzyme digestion, tissue sections are incubated at 37°C for 10-30 minutes in different enzyme solutions such as proteinase K, trypsin, pronase, pepsin etc.

[0202] After rinsing away the epitope retrieval solution or excess enzyme, tissue sections are treated with a blocking buffer to prevent unspecific interactions. The primary antibody is incubated at appropriate dilutions in dilution buffer for 1 hour at room temperature or overnight. Excess primary antibody is rinsed away and sections are incubated in peroxidase blocking solution for 10 min at room temperature. After another washing step, tissue sections are incubated with a secondary antibody labelled with a group that might serve as an anchor for an enzyme. Examples therefore are biotin labelled secondary antibodies that are recognized by streptavidin coupled horseradish peroxidase. Detection of the antibody/enzyme complex is achieved by incubating with a suitable chromogenic substrate.

C. Determination of HER-3 antigen concentration in serum of patients

[0203] A sandwich ELISA is developed to quantify HER-3 levels in human serum. The two fully human monoclonal anti-HER-3 antibodies used in the sandwich ELISA, recognized different domains on the HER-3 molecule and do not compete for binding, for example (*see,* Example 8). The ELISA is performed as follows: 50 μl of capture anti-HER-3 antibody in coating buffer (0.1 M $NaHCO_3$, pH 9.6) at a concentration of 2 μg/ml were coated on ELISA plates (Fisher). After incubation at 4°C overnight, the plates are treated with 200 μl of blocking buffer (0.5 % BSA, 0.1 % Tween 20, 0.01 % Thimerosal in PBS) for 1 hr at 25 °C. The plates were washed (3x) using 0.05 % Tween 20 in PBS (washing buffer, WB). Normal or patient sera (Clinomics, Bioreclaimation) are diluted in blocking buffer containing 50 % human

serum. The plates are incubated with serum samples overnight at 4°C, washed with WB, and then incubated with 100 μl/well of biotinylated detection anti-HER-3 antibody for 1 hr at 25 °C. After washing, the plates are incubated with HRP-Streptavidin for 15 min, washed as before, and then treated with 100 μl/well of o-phenylenediamine in H_2O_2 (Sigma developing solution) for color generation. The reaction is stopped with 50 μl/well of H_2SO_4 (2 M) and analyzed using an ELISA plate reader at 492 nm. The concentration of HER-3 antigen in serum samples is calculated by comparison to dilutions of purified HER-3 antigen using a four parameter curve fitting program.

[0204] Staging of cancer in a patient: Based on the results set forth and discussed under items A, B and C, it is possible to stage a cancer in a subject based on expression levels of the HER-3 antigen. For a given type of cancer, samples of blood are taken from subjects diagnosed as being at various stages in the progression of the disease, and/or at various points in the therapeutic treatment of the cancer. The concentration of the HER-3 antigen present in the blood samples is determined using a method that specifically determines the amount of the antigen that is present. Such a method includes an ELISA method, such as the method described under items A and B. Using a population of samples that provides statistically significant results for each stage of progression or therapy, a range of concentrations of the HER-3 antigen that may be considered characteristic of each stage is designated.

[0205] In order to stage the progression of the cancer in a subject under study, or to characterize the response of the subject to a course of therapy, a sample of blood is taken from the subject and the concentration of the HER-3 antigen present in the sample is determined. The concentration so obtained is used to identify in which range of concentrations the value falls. The range so identified correlates with a stage of progression or a stage of therapy identified in the various populations of diagnosed subjects, thereby providing a stage in the subject under study.

[0206] Anti-HER-3 antibodies as described herein are used for treatment of certain hyperproliferative or HER-3 associated disorders based on a number of factors, such as HER-3 expression, for example. Tumor types such as breast cancer, gastrointestinal cancer, pancreatic cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, kidney cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma, and other HER-3 expressing or overexpressing cancers are examples of indications that are treated with a combination therapy as described herein, although indications are not limited to those in the preceding list. In addition, the following groups of patients may benefit from treatment as described herein:

- Patients not eligible for treatment with anti-HER-2 mAb
- Patients with resistance to anti-HER-1 mAb or small molecule anti-EGF-R inhibitor
- Patients with NSCLC resistant to erlotinib or gefitinib

[0207] Anti-HER-3 antibodies are used in combination with one or more additional agents in a so called "combination therapy." Such combination therapy includes, but is not limited to, the agents disclosed herein. Combination therapy with anti-HER-3 antibodies and other agents can extend patient survival, increase time to tumor progression, or enhance quality of patient life. Protocol and administration design will address therapeutic efficacy as well as the ability to reduce the usual doses of standard therapies, such as chemotherapy or radiation therapy, for example.

[0208] Treatment of humans with anti-HER-3 antibodies: To determine the *in vivo* effects of anti-HER-3 antibody treatment in human patients with tumors, such human patients are injected over a certain amount of time with an effective amount of anti-HER-3 antibody. At periodic times during the treatment, the human patients are monitored to determine whether their tumors progress, in particular, whether the tumors grow and metastasize.

[0209] A tumor patient treated with the anti-HER-3 antibodies has a lower level of tumor growth and/or metastasis compared to the level of tumor growth and metastasis in tumor patients treated with the current standard of care therapeutics.

[0210] Treatment with anti-HER-3 antibody conjugates: To determine the *in vivo* effects of anti-HER-3 antibody conjugates, human patients or animals exhibiting tumors are injected over a certain amount of time with an effective amount of anti-HER-3 antibody conjugate. For example, the anti-HER-3 antibody conjugate administered is DM1-anti-HER-3 antibody conjugate, an auristatin-anti-HER-3 antibody conjugate or radioisotope-anti-HER-3 antibody conjugate. At periodic times during the treatment, the human patients or animals are monitored to determine whether their tumors progress, in particular, whether the tumors grow and metastasize.

[0211] A human patient or animal exhibiting tumors and undergoing treatment with, for example, DM1-anti-HER-3 antibody or radioisotope-anti-HER-3 antibody conjugates has a lower level of tumor growth and metastasis when compared to a control patient or animal exhibiting tumors and undergoing treatment with an alternate therapy. Control DM1-antibodies that may be used in animals include conjugates comprising DM1 linked to antibodies of the same isotype of the anti-HER-3 antibodies, but more specifically, not having the ability to bind to HER-3 tumor antigen. Control radioisotope-antibodies that may be used in animal tests include conjugates comprising radioisotope linked to antibodies of the same isotype of the anti-HER-3 antibodies, but more specifically, not having the ability to bind to HER-3 tumor antigen. Note: the control conjugates would not be administered to humans.

EXAMPLE 33: Identifying First in Human Doses and Schedule of an Anti-HER-3 mAb Based on Preclinical Pharmacokinetic, Pharmacodynamic, and Efficacy Data

[0212] Studies were conducted to use preclinical modeling to predict a minimally effective dose regimen for objective response using preclinical pharmacokinetics (PK), BxPC3 xenograft mice anti-tumor efficacy, and pharmacodynamic (PD) data.

[0213] Mice bearing ~200mm^3 established BxPC3 pancreatic xenografts were treated twice per week with U1-59 at 25, 100, 200, 500 μg/mouse. Inhibition of pHER in the BxPC3 xenograft tumors was analyzed by western blotting. A PK/PD/Efficacy model (based on Simeoni et al. (2004) Cancer Res. 64:1094-1101) was used to prospectively select dose and schedule for further testing. To confirm the PK/PD/Efficacy model, BxPC3 pancreatic tumor-bearing mice were treated with 400 μg/mouse biweekly and 200 μg/mouse biweekly, weekly and twice a week. Interspecies scaling based on body weight (BW) was used to predict U1-59 PK parameters in human on the basis of the serum concentrations obtained in mice, rat and monkeys. The relationship between drug concentration, inhibition of pHER-3 in animals, and interspecies PK scaling was used to select the minimally effective dose for the first in human study.

[0214] U1-59 treatment of BxPC3 xenografts resulted in a statistically significant inhibition of tumor growth and pHER-3 levels in a dose and schedule dependent manner ($p<0.05$). Treatment with U1-59 at 400 μg/mouse biweekly and 200 μg/mouse biweekly, weekly and twice a week resulted in a 50%, 33%, 74% and 70% inhibition of tumor growth ($p<0.05$), a 30%, 58%, 23% and 20% inhibition of pHER-3 (quantitative Western blot) versus the IgG control treated group, respectively. Serum concentrations of U1-59 at necropsy for the respective dose groups were (mean (SD)) of 2.07 (0.97), 0.45 (0.21), 3.08 (0.82) and 34.9 (9.1) μg/mL, respectively. The estimated trough concentration needed to achieve 90% maximal pHER-3 inhibition (IC_{90}) was estimated to be ~3 μg/ml. The PK/PD/efficacy model developed predicted the mean tumor volume ($R^2 = 0.925$). The clearance (CL) and initial volume of distribution (Vd) in man were estimated to be 11 mL/day/kg and 28 mL/kg. Comparison of simulated human PK profiles suggested that biweekly doses of > 3 mg/kg, which should exhibit linear PK, may result in > 90% pHER-3 inhibition during two week dosing interval.

[0215] The anti-tumor efficacy in the BxPC3 pancreatic xenograft model was correlated with an increased serum concentration of U1-59 and a decrease in pHER-3 levels, allowing for development of a PK/PD/Efficacy relationship. This relationship was used to determine a dose and schedule for U1-59 to investigate in a first in human (FIH) study.

EXAMPLE 34: Reactivation Studies

[0216] A549 cells were plated in Ham's F-12 medium (Gibco), all media supplemented with 10% FBS (Hyclone, Logan, UT) and 1X L-glutamine (Gibco). Cells were serum-starved overnight. The media were changed into fresh serum-free media and cells were treated with 50 μg/ml U1-59 or 5 μM gefitinib alone, or combination of U1-59 and gefitinib, for 1 or 24 hours at 37°C. Cells were washed with cold PBS after their respective treatment time points and lysed using RIPA buffer (20 mM Tris-HCl pH 7.5, 1% Igepal, 1% sodium deoxycholate, 150 mM NaCl, 0.1 % SDS, 1% Triton X-100) containing 200 μM phenylmethanesulfonylfluoride (PMSF) (Fluka Biochemica), 200 μM Halt protease inhibitor cocktail kit (Pierce Biotechnology), and 200 μM sodium orthovanadate (Sigma-Aldrich, St. Louis, MO). The lysates were passed through QIA shredder columns (Qiagen) and the flow-through quantitated using a spectrophotometer (Beckman Coulter, Fullerton, CA). Proteins, 50 μg per well, were analyzed in duplicate for pHER3 using ELISA Duoset (R&D systems) according to manufacturer's protocol. The results are shown in FIGURE 20.

OTHER EMBODIMENTS

[0217] It is to be understood that the scope of the invention is defined by the scope of the appended claims.

TABLE 10: CDR Sequences

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-1	235	GGSINSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-1	318	RASQGIRND LG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-2	236	GGSISSGDY YWS	259	YIYYSGSTY YNPSLRS	283	ADYDFWSG YFDY
light	U1-2	318	RASQGIRND LG	343	AASSLQS	361	LQHNGYPW T
heavy	U1-3	237	GGSISSGGY YWS	258	YIYYSGSTY YNPSLKS	284	DGYDSSGY YHGYFDY
light	U1-3	319	KSSQSVLYS SNNKNYLA	344	WASTRES	362	QQYYSTPLT
heavy	U1-4	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-4	318	RASQGIRND LG	343	AASSLQS	363	LQHNNYPW T
heavy	U1-5	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-5	318	RASQGIRND LG	343	AASSLQS	364	LQHNTYPW T

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-6	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	285	ADYDFWNG YFDY
light	U1-6	318	RASQGIRND LG	343	AASSLQS	364	LQHNTYPW T
heavy	U1-7	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-7	320	RASQDIRND LG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-8	238	GYTLTELSM Y	260	GFDPEDGETI YAQKFQG	286	GWNYVFDY
light	U1-8	321	RSSQSLLHS NGYNYLD	345	LDSHRAS	365	MQALQTPLT
heavy	U1-9	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	285	ADYDFWNG YFDY
light	U1-9	320	RASQDIRND LG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-10	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-10	318	RASQGIRND LG	343	AASSLQS	363	LQHNNYPW T

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-11	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-11	318	RASQGIRND LG	343	AASSLQS	364	LQHNTYPW T
heavy	U1-12	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-12	318	RASQGIRND LG	343	AASSLQS	363	LQHNNYPW T
heavy	U1-13	237	GGSISSGGY YWS	258	YIYYSGSTY YNPSLKS	287	EDDGMDV
light	U1-13	322	RSSQSLLHS NGYNYLE	346	LGSNRAS	366	MQALQTPIT
heavy	U1-14	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-14	318	RASQGIRND LG	343	AASSLQS	364	LQHNTYPW T
heavy	U1-15	239	GGSVSSGGY YWS	261	YIYYSGSTN YNPSLKS	288	DGDVDTAM VDAFDI
light	U1-15	323	RASQSLSGN YLA	347	GASSRAT	367	QQYDRSPLT

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-16	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-16	318	RASQGIRND LG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-17	236	GGSISSGDY YWS	262	YIYYSGSTY YNSSLKS	283	ADYDFWSG YFDY
light	U1-17	318	RASQGIRND LG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-18	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	283	ADYDFWSG YFDY
light	U1-18	318	RASQGIRND LG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-19	236	GGSISSGDY YWS	258	YIYYSGSTY YNPSLKS	289	GDYDFWSG EFDY
light	U1-19	sequence not available					
heavy	U1-20	237	GGSISSGGY YWS	263	YIYDSGSTYY NPSLKS	290	DQGQDGYS YGYGYYYG MDV
light	U1-20	324	QASQDISNY LN	348	VASNLET	368	QQCDNLPLT

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-21	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-21	320	RASQDIRNDLG	349	AASRLQS	360	LQHNSYPWT
heavy	U1-22	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-22	318	RASQGIRNDLG	350	AASSLQN	360	LQHNSYPWT
heavy	U1-23	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-23	318	RASQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-24	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	285	ADYDFWNGYFDY
light	U1-24	318	RASQGIRNDLG	343	AASSLQS	363	LQHNNYPWT
heavy	U1-25	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-25	318	RASQGIRNDLG	350	AASSLQN	360	LQHNSYPWT

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-26	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	291	ADYDFWSGYFDF
light	U1-26	318	RASQGIRNDLG	343	AASSLQS	361	LQHNGYPWT
heavy	U1-27	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	291	ADYDFWSGYFDF
light	U1-27	318	RASQGIRNDLG	343	AASSLQS	361	LQHNGYPWT
heavy	U1-28	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	292	ADYDFWSGYFDS
light	U1-28	318	RASQGIRNDLG	343	AASSLQS	361	LQHNGYPWT
heavy	U1-29	240	GFTFNSYDMH	264	VIWYDGSNKYYADSVKG	293	DRLCTNGVCYEDYGMDV
light	U1-29	324	QASQDISNYLN	351	DASNLET	369	QHYDTLPLT
heavy	U1-30	236	GGSISSGDYYWS	265	YIYYSGTTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-30	325	RAGQGIRNDLG	343	AASSLQS	360	LQHNSYPWT

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-31	241	GYTFTNYGIS	266	WISAYDGYRNYAQKLQG	294	DVQDYGDYDYFDY
light	U1-31	326	RASQSISSYLN	343	AASSLQS	370	QQSYSTPIT
heavy	U1-32	236	GGSISSGDYYWS	265	YIYYSGTTYYNPSLKS	283	ADYDFWSGYFDY
light	U1-32	325	RAGQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-33	236	GGSISSGDYYWS	258	YIYYSGSTYYNPSLKS	295	ADYDFWSGHFDC
light	U1-33	327	RASQGIRDDLG	352	AESSLQS	371	LQHHSYPWT
heavy	U1-34	241	GYTFTNYGIS	266	WISAYDGYRNYAQKLQG	294	DVQDYGDYDYFDY
light	U1-34	326	RASQSISSYLN	343	AASSLQS	370	QQSYSTPIT
heavy	U1-35	242	GFTFSDYYMS	267	YISSSGNNIYHADSVKG	296	ERYSGYDDPDGFDI
light	U1-35	328	QASQDISNYLS	351	DASNLET	372	QQYDNPPCS

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-36	243	GGSISSGYYYWS	268	YIYYSGTTYYNPSFKS	297	ADYDFWSGHFDY
light	U1-36	318	RASQGIRNDLG	343	AASSLQS	360	LQHNSYPWT
heavy	U1-37	244	GYTFTSYGIS	269	WISAYDGHTNYAQKLQG	298	DPHDYSNYEAFDF
light	U1-37	326	RASQSISSYLN	343	AASSLQS	370	QQSYSTPIT
heavy	U1-38	245	GFSLSTSGVGVG	270	LIYWNDDKRYSPSLKS	299	RDEVRGFDY
light	U1-38	329	RSSQSLVYSDGYTYLH	353	KVSNWDS	373	MQGAHWPIT
heavy	U1-39	246	GFTVSSNYMS	271	VIYSGGSTYYADSVKG	300	GQWLDV
light	U1-39	321	RSSQSLLHSNGYNYLD	354	LGFHRAS	374	RQALQTPLT
heavy	U1-40	237	GGSISSGGYYWS	272	YIYSSGSTYYNPSLKS	301	DRELELYYYYYGMDV
light	U1-40	330	RSSQSLLYSNGYNYLD	346	LGSNRAS	365	MQALQTPLT

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-41	237	GGSISSGGGY YWS	258	YIYYSGSTYY NPSLKS	302	DRELEGYSN YYGVDV
light	U1-41	331	RASQAISNY LN	343	AASSLQS	375	QQNNSLPIT
heavy	U1-42	247	GYSFTSYWI G	273	IIYPGDSDTR YSPSFQG	303	HENYGDYN Y
light	U1-42	332	RASQSIRSYL N	343	AASSLQS	376	QQSNGSPLT
heavy	U1-43	237	GGSISSGGGY YWS	259	YIYYSGSTYY NPSLRS	304	DREREWDD YGDPQGMD V
light	U1-43	333	RASQSISSYL H	343	AASSLQS	377	QQSYSNPLT
heavy	U1-44	247	GYSFTSYWI G	274	IIWPGDSDTI YSPSFQG	303	HENYGDYN Y
light	U1-44	332	RASQSIRSYL N	343	AASSLQS	378	QQSISSPLT
heavy	U1-45	248	GYTFTSYDI N	275	WMNPNSGDT GYAQVFQG	305	FGDLPYDYS YYEWFDP
light	U1-45	326	RASQSISSYL N	343	AASSLQS	379	QQSYSTPLT

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-46	249	GDSVSSNSAAWN	276	RTYYRSKWYNDYAVSVKS	306	DLYDFWSGYPYYYGMDV
light	U1-46	sequence not available					
heavy	U1-47	249	GDSVSSNSAAWN	276	RTYYRSKWYNDYAVSVKS	307	DYYGSGSFYYYYGMDV
light	U1-47	326	RASQSISSYLN	355	AASNLQS	380	QQSYSTPRT
heavy	U1-48	250	GGSISSYYWS	277	HIYTSGSTNYNPSLKS	308	EAIFGVGPYYYYGMDV
light	U1-48	sequence not available					
heavy	U1-49	251	GYTFTGYYMH	278	WINPNIGGTNCAQKFQG	309	GGRYSSSWSYYYYGMDV
light	U1-49	334	KSSQSLLLSDGGTYLY	356	EVSNRFS	381	MQSMQLPIT
heavy	U1-50	239	GGSVSSGGYYWS	261	YIYYSGSTNYNPSLKS	310	GGDSNYEDYYYYYGMDV
light	U1-50	335	RASQSISIYLH	343	AASSLQS	382	QQSYTSPIT

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-51	250	GGSISSYYWS	261	YIYYSGSTNYNPSLKS	311	DSSYYDSSGYYLYYYAMDV
light	U1-51	319	KSSQSVLYSSNNKNYLA	344	WASTRES	383	QQYYTTPLT
heavy	U1-52	237	GGSISSGGYYWS	279	NIYYSGSTYYNPSLKS	312	GGTGTNYYYYYGMDV
light	U1-52	336	RASQSVSSSYLA	357	GASSWAT	384	QQYGSSPLT
heavy	U1-53	252	GFTFSIYSMN	280	YISSSSSTIYYADSVKG	313	DRGDFDAFDI
light	U1-53	337	QASQDITNYLN	351	DASNLET	385	QQCENFPIT
heavy	U1-55.1	253	GGSVSSGGYYWN	281	YINYSGSTNYNPSLKS	301	DRELELYYYYYGMDV
light	U1-55.1	same as U1-55					
heavy	U1-55	will be same as U1-55.1					
light	U1-55	338	RSSQSLLYSNGYKYLD	346	LGSNRAS	366	MQALQTPIT
heavy	U1-57.1	same as U1-57					
light	U1-57.1	338	RSSQSLLYSNGYKYLD	346	LGSNRAS	366	MQALQTPIT

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
heavy	U1-57	254	GGSVSSGGYYWN	281	YINYSGSTNYNPSLKS	301	DRELELYYYYYGMDV
light	U1-57	will be same as U1-57.1					
heavy	U1-58	255	GFTFSSYGMH	264	VIWYDGSNKYYADSVKG	314	AARLDYYYGMDV
light	U1-58	339	RASQSINSYLN	358	GASGLQS	386	QQSYSSPLT
heavy	U1-59	256	GGSFSGYYWS	282	EINHSGSTNYNPSLKS	315	DKWTWYFDL
light	U1-59	340	RSSQSVLYSSSNRNYLA	344	WASTRES	387	QQYYSTPRT
heavy	U1-61.1	257	GVSISSGGYYWS	258	YIYYSGSTYYNPSLKS	316	DSESEYSSSSNYGMDV
light	U1-61.1	same as U1-61.1					
heavy	U1-61	257	GVSISSGGYYWS	258	YIYYSGSTYYNPSLKS	316	DSESEYSSSSNYGMDV
light	U1-61	341	RASQTISSYLN	359	AASSLQG	377	QQSYSNPLT
heavy	U1-62	247	GYSFTSYWIG	273	IIYPGDSDTRYSPSFQG	317	QMAGNYYYGMDV

(continued)

AB-Chain	PAT ID:	SEQ ID:	CDR1	SEQ ID:	CDR2	SEQ ID:	CDR3
light	U1-62	342	RASQSVISIY LA	347	GASSRAT	388	QQYGSSPCS

Claims

1. A first agent and a second agent for use in the treatment or prevention of a hyperproliferative disease associated with HER-3,
wherein said hyperproliferative disease is HER-3 expressing or overexpressing cancer,
wherein said second agent is trastuzumab, and
wherein said first agent is an antigen-binding protein that binds to HER-3, and comprises: a heavy chain amino acid sequence that comprises a CDRH1 as shown in SEQ ID NO: 256; a CDRH2 as shown in SEQ ID NO: 282; and a CDRH3 as shown in SEQ ID NO: 315; and a light chain amino acid sequence that comprises a CDRL1 as shown in SEQ ID NO: 340; a CDRL2 as shown in SEQ ID NO: 344; and a CDRL3 as shown in SEQ ID NO: 387.

2. The first agent and the second agent for the use of claim 1, wherein said first agent is an antigen-binding protein that binds to HER-3, and comprises a heavy chain amino acid sequence as shown in SEQ ID NO: 70 or/and a light chain amino acid sequence as shown in SEQ ID NO: 72.

3. The first agent and the second agent for the use of claim 1, wherein said first agent is an antigen-binding protein that binds to HER-3, and comprises the heavy chain amino acid sequence of SEQ ID NO: 70 and the light chain amino acid sequence of SEQ ID NO: 72.

4. The first agent and the second agent for the use of any one of claims 3 to 6, wherein said antigen-binding protein is directed against the extracellular domain of HER-3, or reduces HER-3-mediated signal transduction, or reduces HER-3 phosphorylation, or reduces cell proliferation, or reduces cell migration or/and increases the downregulation of HER-3.

5. The first agent and the second agent for the use of any one of the preceding claims, wherein said antigen-binding protein that binds to HER-3 is an antibody, particularly a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof, particularly a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fv fragment, a diabody, or a single chain antibody molecule, particularly an antibody of the IgG1-, IgG2-, IgG3- or IgG4- type.

6. The first agent and the second agent for the use of claim 1, wherein said first agent is coupled to an effector group, particularly to a radioisotope or radionuclide, a toxin, or a therapeutic or chemotherapeutic group, wherein the therapeutic or chemotherapeutic group is preferably calicheamicin, auristatinPE, geldanamycin, maytansine and derivatives thereof.

7. The first agent and the second agent for the use of claim 1, wherein said treatment or prevention comprises administering a further therapeutic agent and/or radiation therapy, wherein the further therapeutic agent is preferably an anti-neoplastic agent or an anti-tumor antibody or a chemotherapeutic agent, wherein the chemotherapeutic agent is preferably selected from the group consisting of capecitabine, anthracycline, doxorubicin, cyclophosphamide, paclitaxel, docetaxel, cisplatin, gemcitabine, and carboplatin.

8. The first agent and the second agent for the use of claim 1, wherein said first agent and said second agent are for administration by intravenous, subcutaneous, intramuscular or oral administration.

9. The first agent and the second agent for the use of claim 1, wherein said hyperproliferative disease is selected from the group consisting of breast cancer, ovarian cancer, prostate cancer, colon cancer, renal cancer, lung cancer, pancreatic cancer, epidermoid carcinoma, fibrosarcoma, melanoma, nasopharyngeal carcinoma, and squamous cell carcinoma.

10. The first agent and the second agent for the use of claim 1, said treatment or prevention comprising administering said first agent or second agent at a dose of about 1 to about 20 mg/kg body weight, at least once every 6 weeks.

11. The first agent and the second agent for the use of claim 1, said treatment or prevention further comprising, after the administration to a subject, monitoring the therapeutic outcome.

Patentansprüche

1. Ein erster Wirkstoff und ein zweiter Wirkstoff zur Verwendung in der Behandlung oder Prävention einer hyperproliferativen Erkrankung im Zusammenhang mit HER-3,
wobei die hyperproliferative Erkrankung HER-3 exprimierender oder überexprimierender Krebs ist,
wobei der zweite Wirkstoff trastuzumab ist, und
wobei der erste Wirkstoff ein Antigen-bindendes Protein ist, das an HER-3 bindet und umfasst: eine schwerkettige Aminosäuresequenz, die ein CDRH1 wie in SEQ ID NO: 256 gezeigt; ein CDRH2 wie in SEQ ID NO: 282 gezeigt; und ein CDRH3 wie in SEQ ID NO: 315 gezeigt, umfasst; und eine leichtkettige Aminosäuresequenz, die ein CDRL1 wie in SEQ ID NO: 340 gezeigt; ein CDRL2 wie in SEQ ID NO: 344 gezeigt; und ein CDRL3 wie in SEQ ID NO: 387 gezeigt, umfasst.

2. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach Anspruch 1, wobei der erste Wirkstoff ein Antigen-bindendes Protein ist, das an HER-3 bindet, und eine schwerkettige Aminosäuresequenz wie in SEQ ID NO: 70 gezeigt oder/und eine leichtkettige Aminosäuresequenz wie in SEQ ID NO: 72 gezeigt, umfasst.

3. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach Anspruch 1, wobei der erste Wirkstoff ein Antigen-bindendes Protein ist, das an HER-3 bindet und die schwerkettige Aminosäuresequenz von SEQ ID NO: 70 und die leichtkettige Aminosäuresequenz von SEQ ID NO: 72 umfasst.

4. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach einem der Ansprüche 3 bis 6, wobei das Antigen-bindende Protein gegen die extrazelluläre Domäne von HER-3 gerichtet ist, oder HER-3-vermittelte Signalübertragung reduziert, oder HER-3-Phosphorylierung reduziert, oder Zellproliferation reduziert oder Zellmigration reduziert oder/und Downregulation von HER-3 erhöht.

5. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach einem der vorhergehenden Ansprüche, wobei das Antigen-bindende Protein, das an HER-3 bindet, ein Antikörper ist, insbesondere ein monoklonaler Antikörper, ein polyklonaler Antikörper, ein rekombinanter Antikörper, ein humanisierter Antikörper, ein humaner Antikörper, ein chimärer Antikörper, ein multispezifischer Antikörper, oder ein Antikörperfragment davon, insbesondere ein Fab Fragment, ein Fab' Fragment, ein F(ab')2 Fragment, ein Fv Fragment, ein Diabody, oder ein Einzelketten-Antikörper-Molekül, insbesondere ein Antikörper des IgG1-, IgG2-, IgG3- oder IgG4-Typs.

6. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach Anspruch 1, wobei der erste Wirkstoff an eine Effektorgruppe gekoppelt ist, insbesondere an ein Radioisotop oder Radionuklid, ein Toxin oder eine therapeutische oder chemotherapeutische Gruppe, wobei die therapeutische oder chemotherapeutische Gruppe vorzugsweise Calicheamicin, AuristatinPE, Geldanamycin, Maytansin und Derivate davon ist.

7. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach Anspruch 1, wobei die Behandlung oder Prävention Verabreichen eines weiteren therapeutischen Wirkstoffs und/oder Bestrahlungstherapie umfasst, wobei der weitere therapeutische Wirkstoff vorzugsweise ein antineoplastischer Wirkstoff oder ein anti-Tumor-Antikörper oder ein chemotherapeutischer Wirkstoff ist, wobei der chemotherapeutische Wirkstoff vorzugsweise ausgewählt ist aus der Gruppe bestehend aus Capecitabin, Anthracyclin, Doxorubicin, Cyclophosphamid, Paclitaxel, Docetaxel, Cisplatin, Gemcitabin, und Carboplatin.

8. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach Anspruch 1, wobei der erste Wirkstoff und der zweite Wirkstoff zur Verabreichung mittels intravenöser, subkutaner, intramuskulärer oder oraler Verabreichung sind.

9. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach Anspruch 1, wobei die hyperproliferative Erkrankung ausgewählt ist aus der Gruppe bestehend aus Brustkrebs, Eierstockkrebs, Prostatakrebs, Darmkrebs, Nierenkrebs, Lungenkrebs, Pankreaskrebs, Epidermoidkarzinom, Fibrosarkom, Melanom, Nasopharynxkarzinom und Plattenepithelkarzinom.

10. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach Anspruch 1, wobei die Behandlung oder Prävention Verabreichen des ersten Wirkstoffs oder zweiten Wirkstoffs in einer Dosis von ungefähr 1 bis ungefähr 20 mg/kg Körpergewicht, mindestens einmal alle 6 Wochen, umfasst.

11. Der erste Wirkstoff und der zweite Wirkstoff zur Verwendung nach Anspruch 1, wobei die Behandlung oder Prävention weiter umfasst, nach der Verabreichung an ein Subjekt, Überwachen des Therapieergebnisses.

Revendications

1. Premier agent et second agent pour une utilisation dans le traitement ou la prévention d'une maladie hyperproliférative associée au HER-3,
dans lesquels ladite maladie hyperproliférative est un cancer exprimant ou surexprimant le HER-3,
dans lesquels ledit second agent est le trastuzumab, et
dans lesquels ledit premier agent est une protéine de liaison d'antigène qui se lie au HER-3, et comprend : une séquence d'acides aminés de chaîne lourde qui comprend une CDRH1 telle que représentée par SEQ ID NO : 256 ; une CDRH2 telle que représentée par SEQ ID NO : 282 ; et une CDRH3 telle que représentée par SEQ ID NO : 315 ; et une séquence d'acides aminés de chaîne légère qui comprend une CDRL1 telle que représentée par SEQ ID NO : 340 ; une CDRL2 telle que représentée par SEQ ID NO : 344 ; et une CDRL3 telle que représentée par SEQ ID NO : 387.

2. Premier agent et second agent pour l'utilisation selon la revendication 1, dans lesquels ledit premier agent est une protéine de liaison d'antigène qui se lie au HER-3, et comprend une séquence d'acides aminés de chaîne lourde telle que représentée par SEQ ID NO : 70 ou/et une séquence d'acides aminés de chaîne légère telle que représentée par SEQ ID NO : 72.

3. Premier agent et second agent pour l'utilisation selon la revendication 1, dans lesquels ledit premier agent est une protéine de liaison d'antigène qui se lie au HER-3, et comprend la séquence d'acides aminés de chaîne lourde de SEQ ID NO : 70 et la séquence d'acides aminés de chaîne légère de SEQ ID NO : 72.

4. Premier agent et second agent pour l'utilisation selon l'une quelconque des revendications 3 à 6, dans lesquels ladite protéine de liaison d'antigène est dirigée contre le domaine extracellulaire du HER-3, ou réduit la transduction des signaux médiée par le HER-3, ou réduit la phosphorylation du HER-3, ou réduit la prolifération cellulaire, ou réduit la migration cellulaire ou/et augmente la régulation négative du HER-3.

5. Premier agent et second agent pour l'utilisation selon l'une quelconque des revendications précédentes, dans lesquels ladite protéine de liaison d'antigène qui se lie au HER-3 est un anticorps, particulièrement un anticorps monoclonal, un anticorps polyclonal, un anticorps recombinant, un anticorps humanisé, un anticorps humain, un anticorps chimérique, un anticorps multispécifique, ou l'un de leurs fragments d'anticorps, particulièrement un fragment Fab, un fragment Fab', un fragment F(ab')2, un fragment Fv, un diacorps, ou une molécule d'anticorps monocaténaire, particulièrement un anticorps du type IgG1, IgG2, IgG3 ou IgG4.

6. Premier agent et second agent pour l'utilisation selon la revendication 1, dans lesquels ledit premier agent est couplé à un groupe effecteur, particulièrement à un radio-isotope ou un radionucléide, une toxine, ou un groupe thérapeutique ou chimiothérapeutique, dans lesquels le groupe thérapeutique ou chimiothérapeutique est de préférence la calichéamicine, l'auristatine-PE, la geldanamycine, la maytansine et leurs dérivés.

7. Premier agent et second agent pour l'utilisation selon la revendication 1, dans lesquels ledit traitement ou ladite prévention comprend l'administration d'un autre agent thérapeutique et/ou d'une radiothérapie, dans lesquels l'autre agent thérapeutique est de préférence un agent antinéoplasique ou un anticorps antitumoral ou un agent chimiothérapeutique, dans lesquels l'agent chimiothérapeutique est choisi de préférence dans le groupe constitué de capécitabine, anthracycline, doxorubicine, cyclophosphamide, paclitaxel, docétaxel, cisplatine, gemcitabine, et carboplatine.

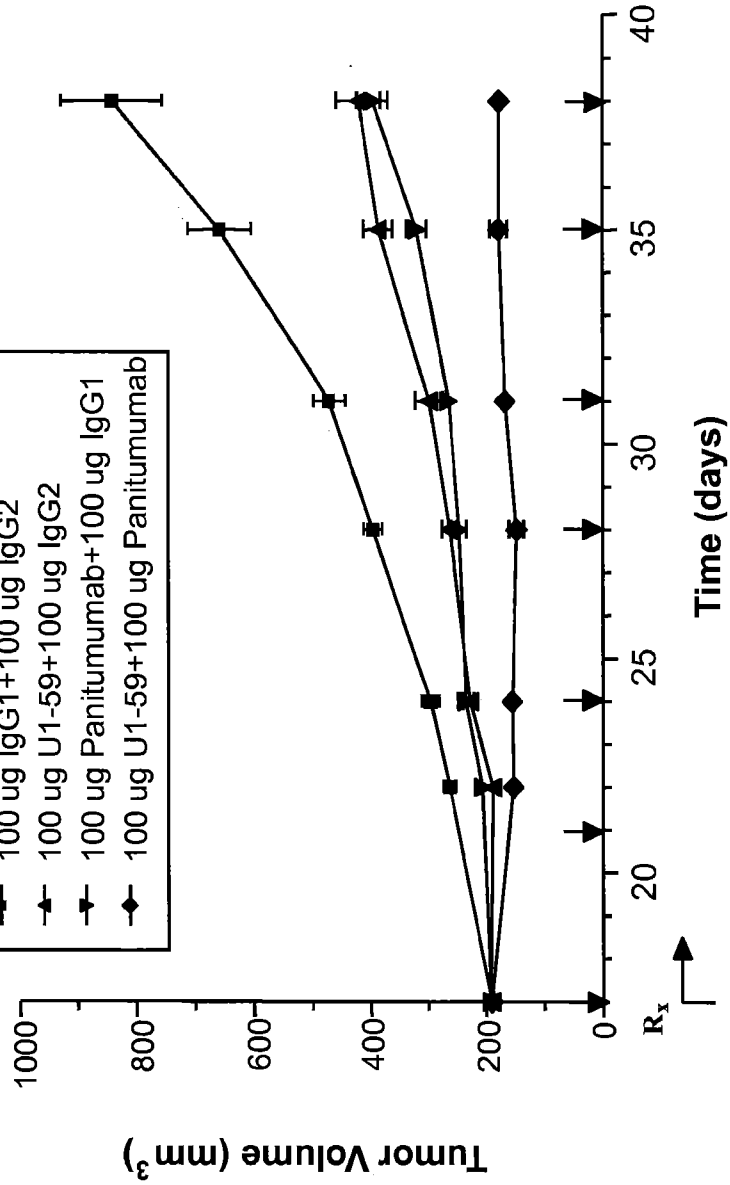
8. Premier agent et second agent pour l'utilisation selon la revendication 1, dans lesquels ledit premier agent et ledit second agent sont pour une administration par administration intraveineuse, sous-cutanée, intramusculaire ou orale.

9. Premier agent et second agent pour l'utilisation selon la revendication 1, dans lesquels ladite maladie hyperproliférative est choisie dans le groupe constitué de cancer du sein, cancer ovarien, cancer de la prostate, cancer du côlon, cancer rénal, cancer du poumon, cancer pancréatique, carcinome épidermoïde, fibrosarcome, mélanome, carcinome nasopharyngé, et carcinome à cellules squameuses.

10. Premier agent et second agent pour l'utilisation selon la revendication 1, ledit traitement ou ladite prévention comprenant l'administration dudit premier agent ou second agent à une dose d'environ 1 à environ 20 mg/kg de poids corporel, au moins une fois toutes les 6 semaines.

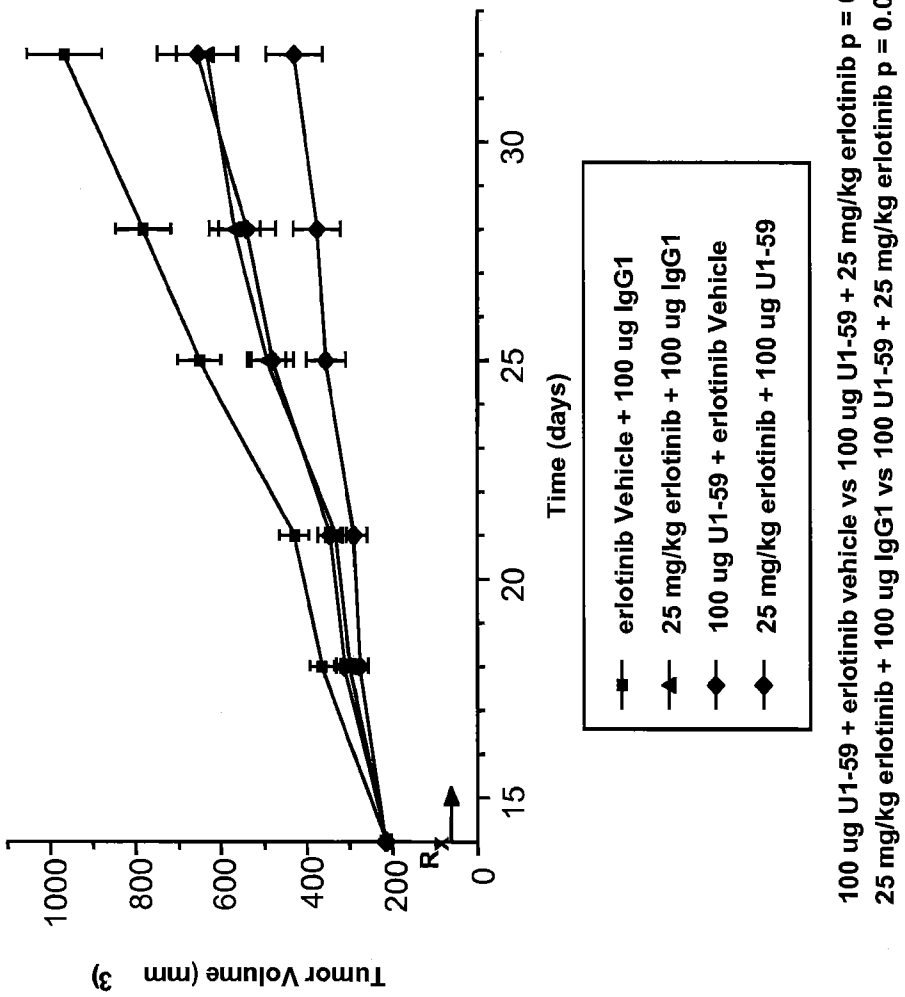
11. Premier agent et second agent pour l'utilisation selon la revendication 1, ledit traitement ou ladite prévention comprenant en outre, après l'administration à un sujet, la surveillance du résultat thérapeutique.

Figure 1



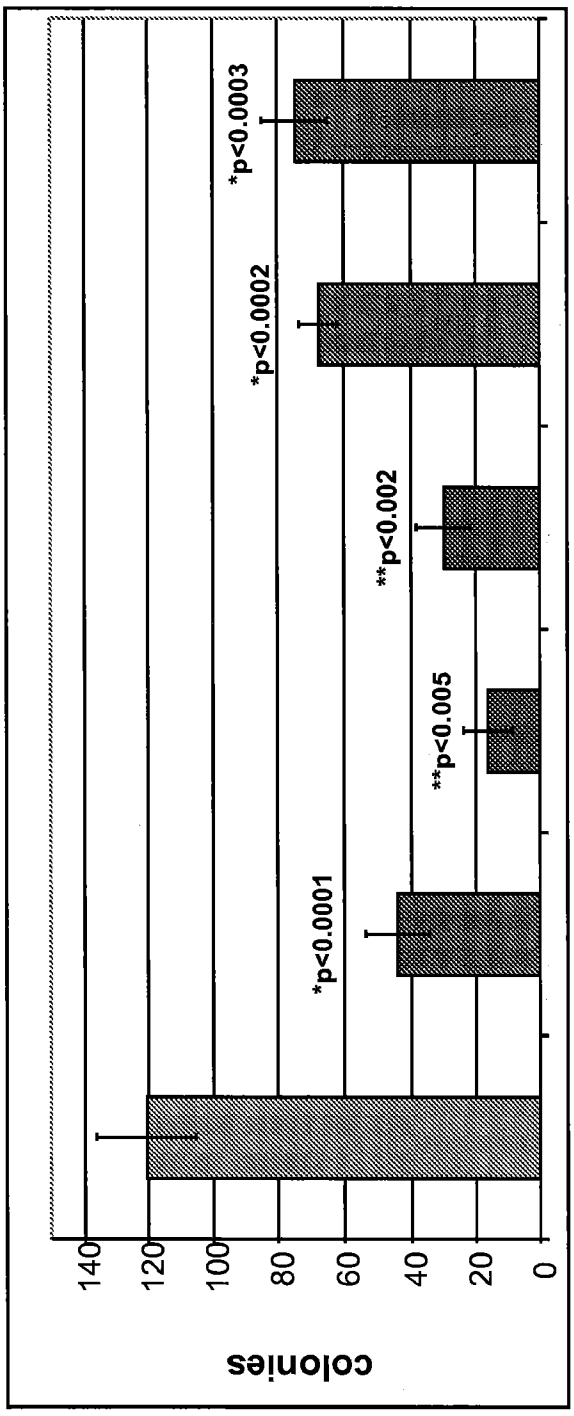
U1-59+IgG2 vs U1-59+Panitumumab $p < 0.0001$
Panitumumab+IgG1 vs U1-59+Panitumumab $p < 0.0001$

Figure 2



100 ug U1-59 + erlotinib vehicle vs 100 ug U1-59 + 25 mg/kg erlotinib p = 0.0376
25 mg/kg erlotinib + 100 ug IgG1 vs 100 U1-59 + 25 mg/kg erlotinib p = 0.0514

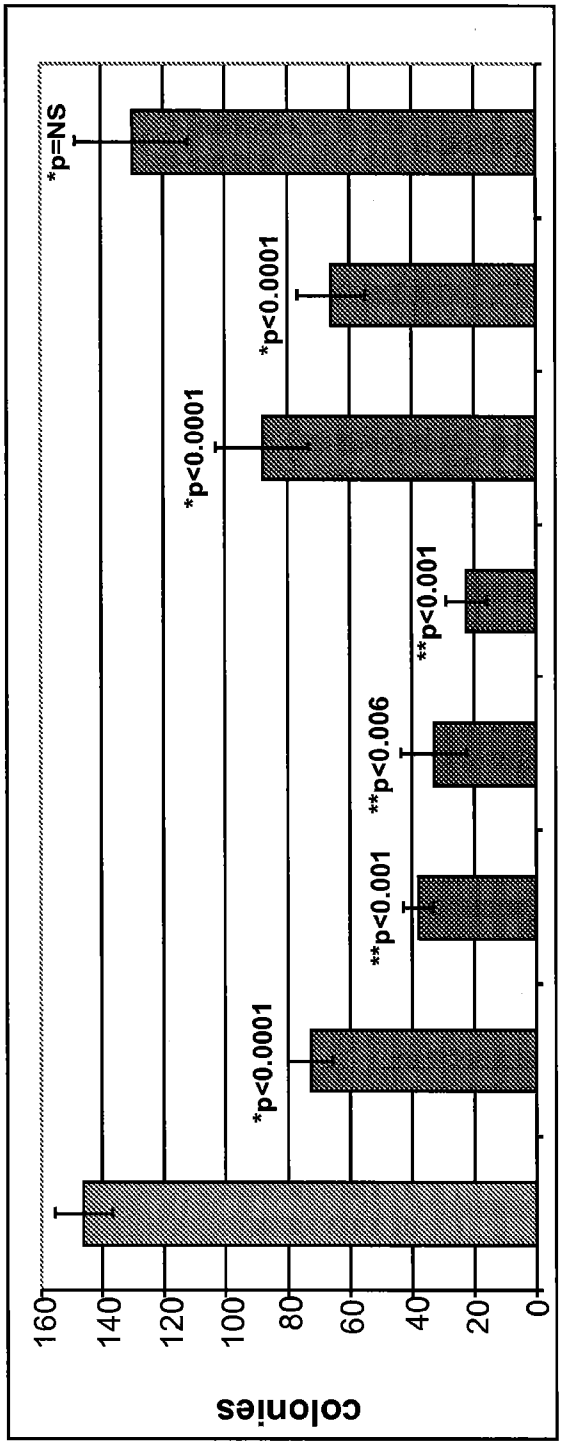
Figure 3



IgG	10***	5			5	5
U1-59		5	5			
c2C4			5		5	
trastuzumab				5		5

* = versus control treatment
** = versus either single agent alone
NS = not significant
*** = μg/ml

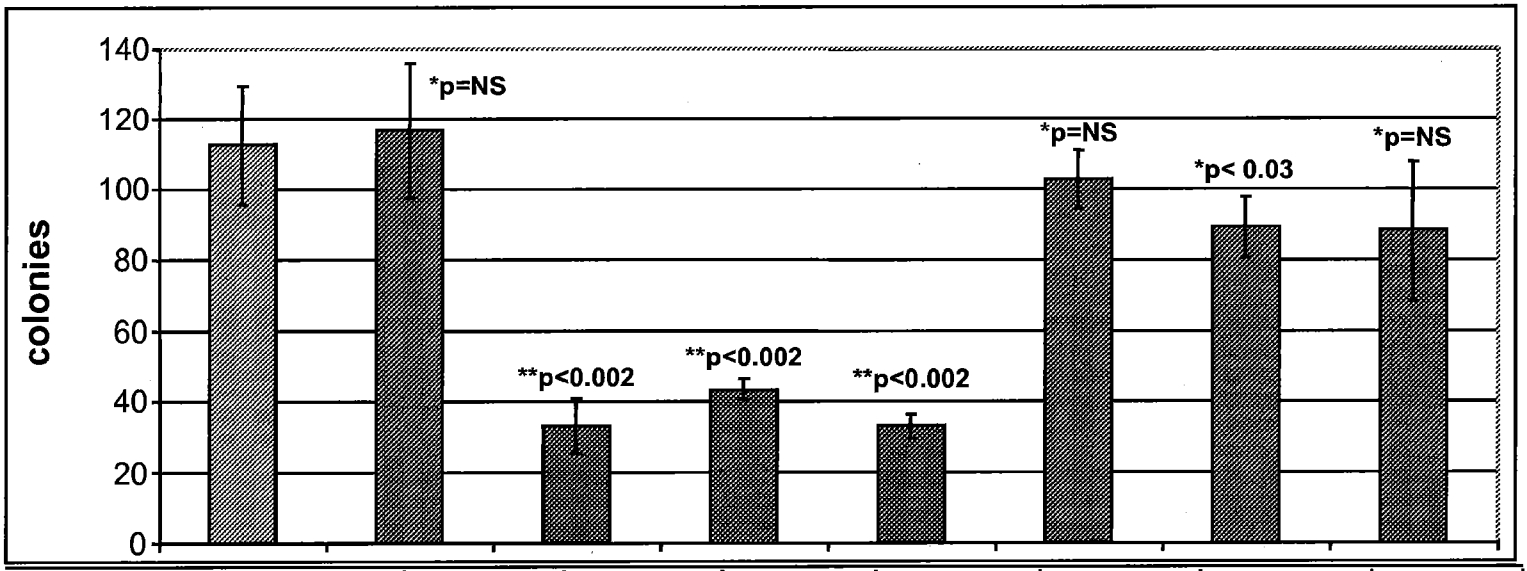
Figure 4



IgG	10***	5				5	5	5	5
U1-59		5	5	5					
c2C4				5			5		
trastuzumab						5			5
cetuximab			5					5	

* = versus control treatment
** = versus either single agent alone
NS = not significant
*** = μg/ml

Figure 5



IgG	10***	7	4	4	4	7	7	7
U1-59		3	3	3	3			
c2C4				3			3	
trastuzumab					3			3
cetuximab			3			3		

* = versus control treatment
** = versus either single agent alone
NS = not significant
*** = μg/ml

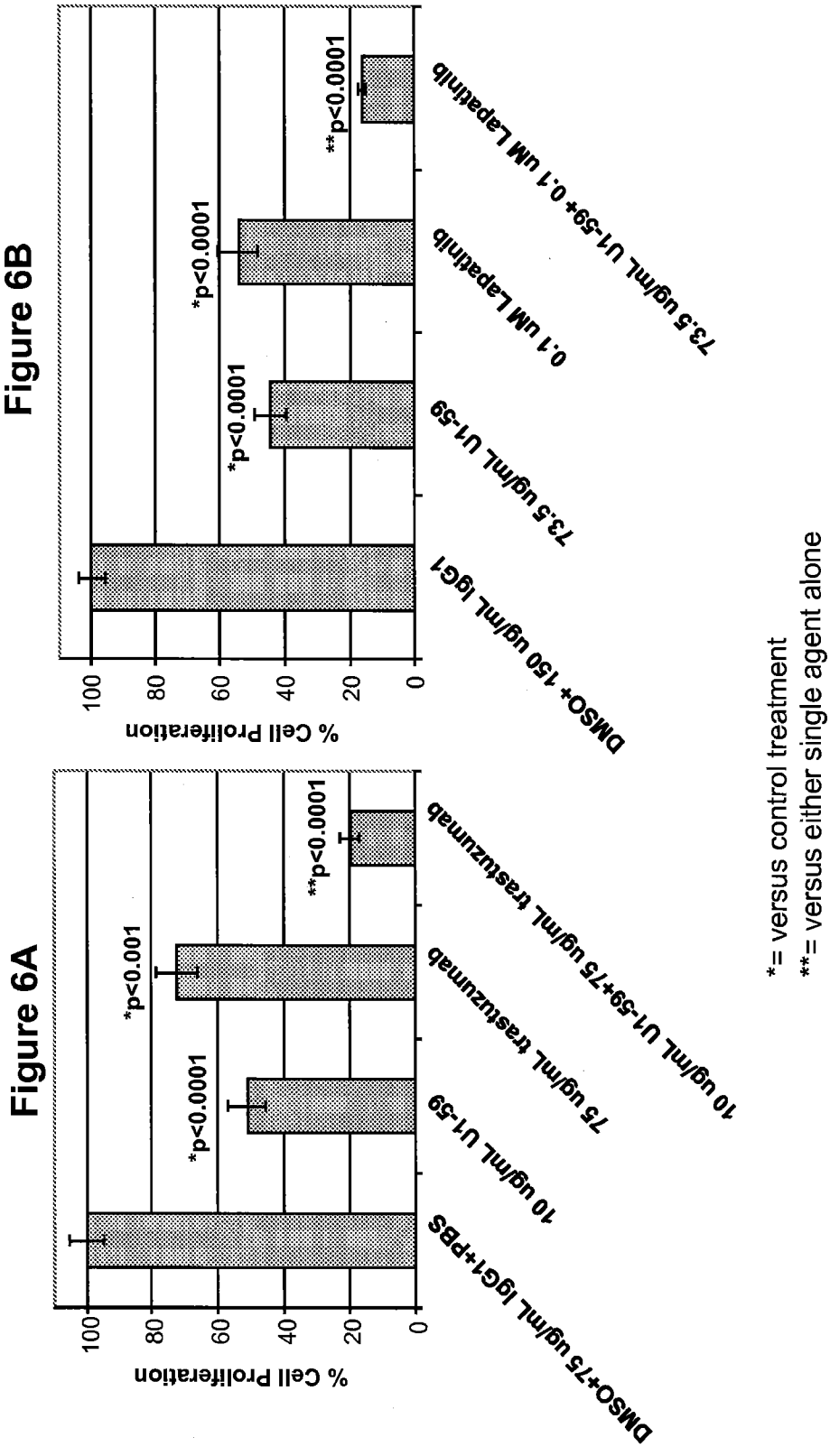


Figure 6B
% Cell Proliferation
0
20
40
60
80
100
*p<0.0001
*p<0.0001
**p<0.0001
DMSO+150 ug/mL IgG1
73.5 ug/mL U1-59
0.1 uM Lapatinib
73.5 ug/mL U1-59+ 0.1 uM Lapatinib
Figure 6A
% Cell Proliferation
0
20
40
60
80
100
*p<0.0001
*p<0.001
**p<0.0001
DMSO+75 ug/mL IgG1+PBS
10 ug/mL U1-59
75 ug/mL trastuzumab
10 ug/mL U1-59+75 ug/mL trastuzumab
*= versus control treatment
**= versus either single agent alone

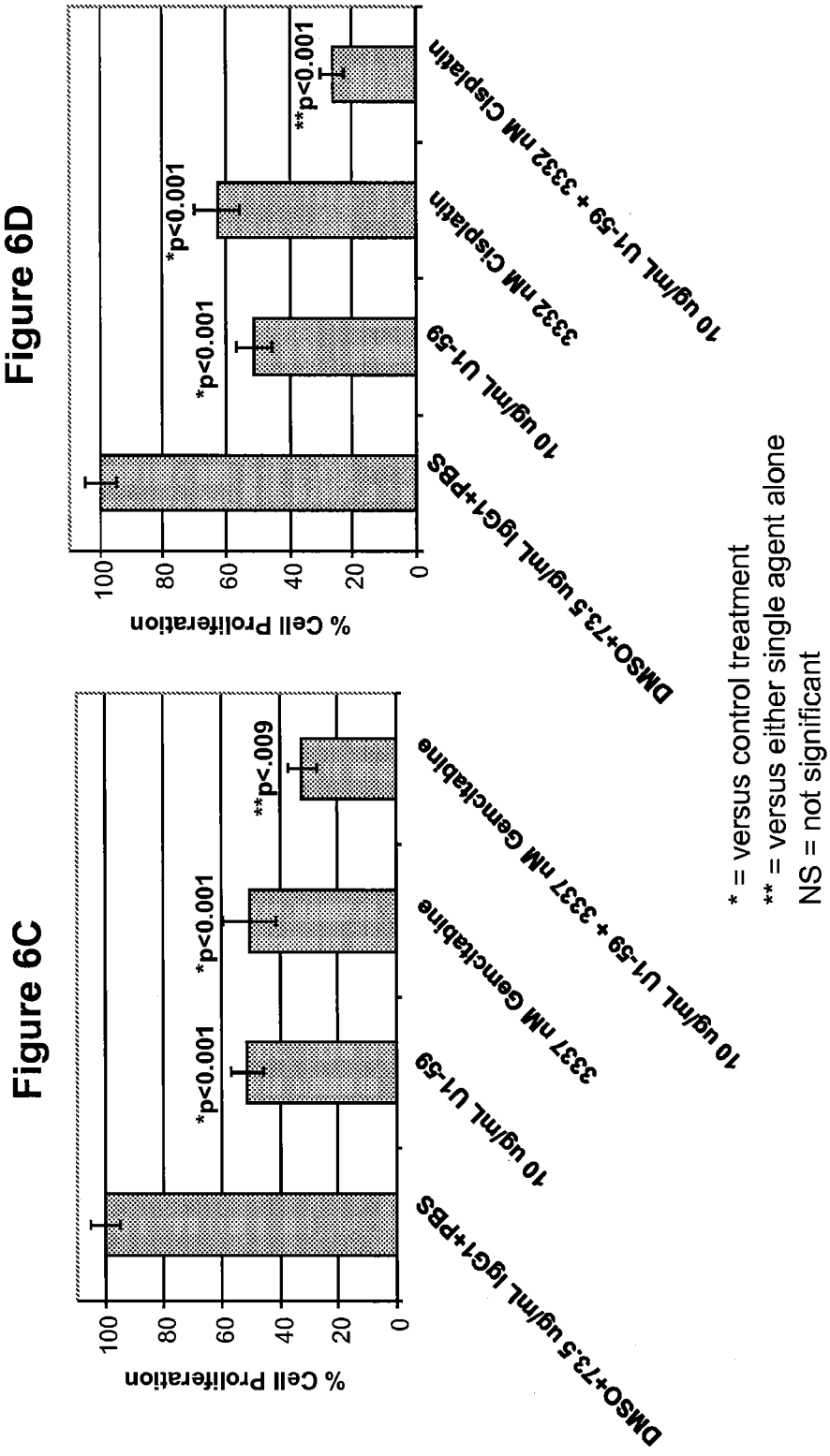
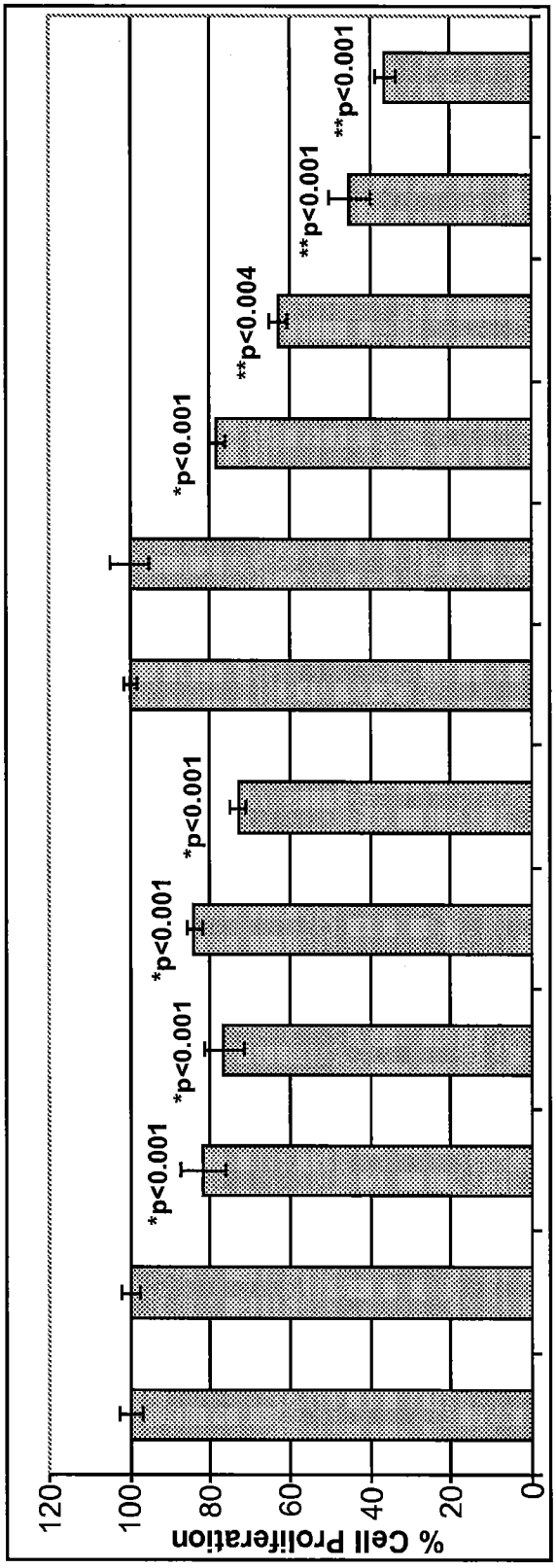


Figure 6C
% Cell Proliferation
100
80
60
40
20
0
*p<0.001
*p<0.001
**p<.009
DMSO+73.5 ug/mL IgG1+PBS
10 ug/mL U1-59
3337 nM Gemcitabine
10 ug/mL U1-59 + 3337 nM Gemcitabine
Figure 6D
% Cell Proliferation
100
80
60
40
20
0
*p<0.001
*p<0.001
**p<0.001
DMSO+73.5 ug/mL IgG1+PBS
10 ug/mL U1-59
3332 nM Cisplatin
10 ug/mL U1-59 + 3332 nM Cisplatin
* = versus control treatment
** = versus either single agent alone
NS = not significant

Figure 7



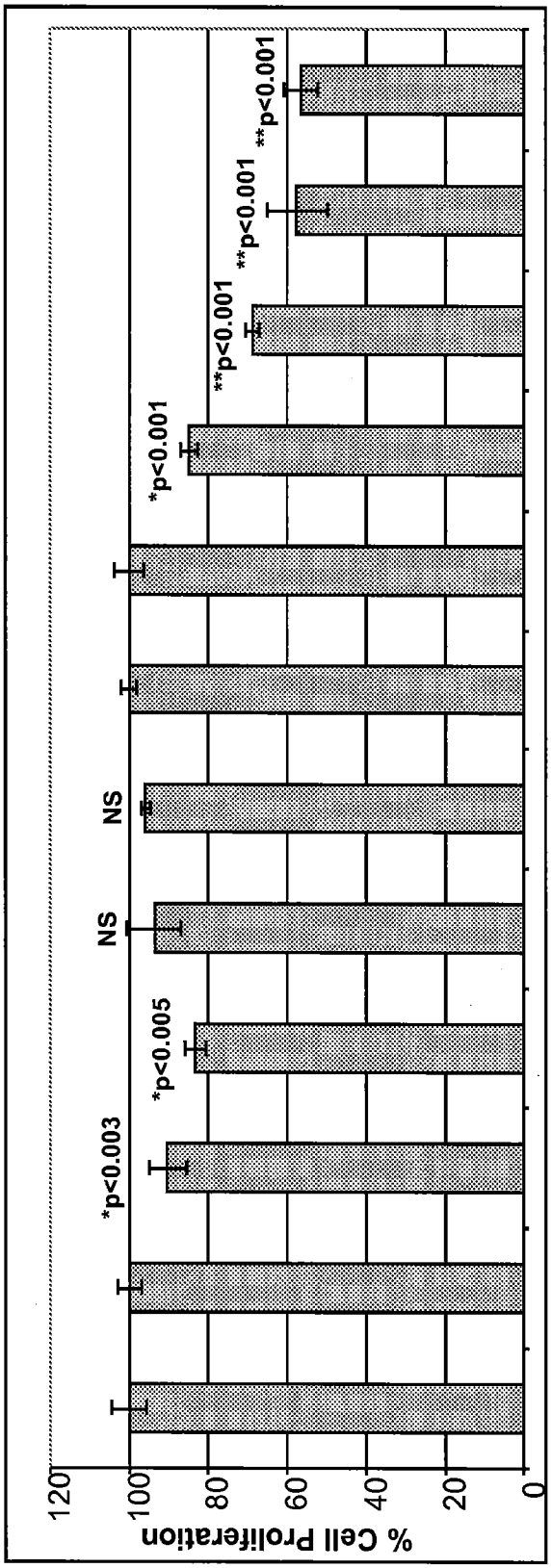
IgG	10***							20	10	10	10			
DMSO		+							+					
U1-59			10							10	10	10	10	
c2C4				10							10			
trastuzumab					10							10		
Lapatinib (0.1 μM)								+						+

* = versus control treatment

** = versus either single agent alone

*** = μg/ml

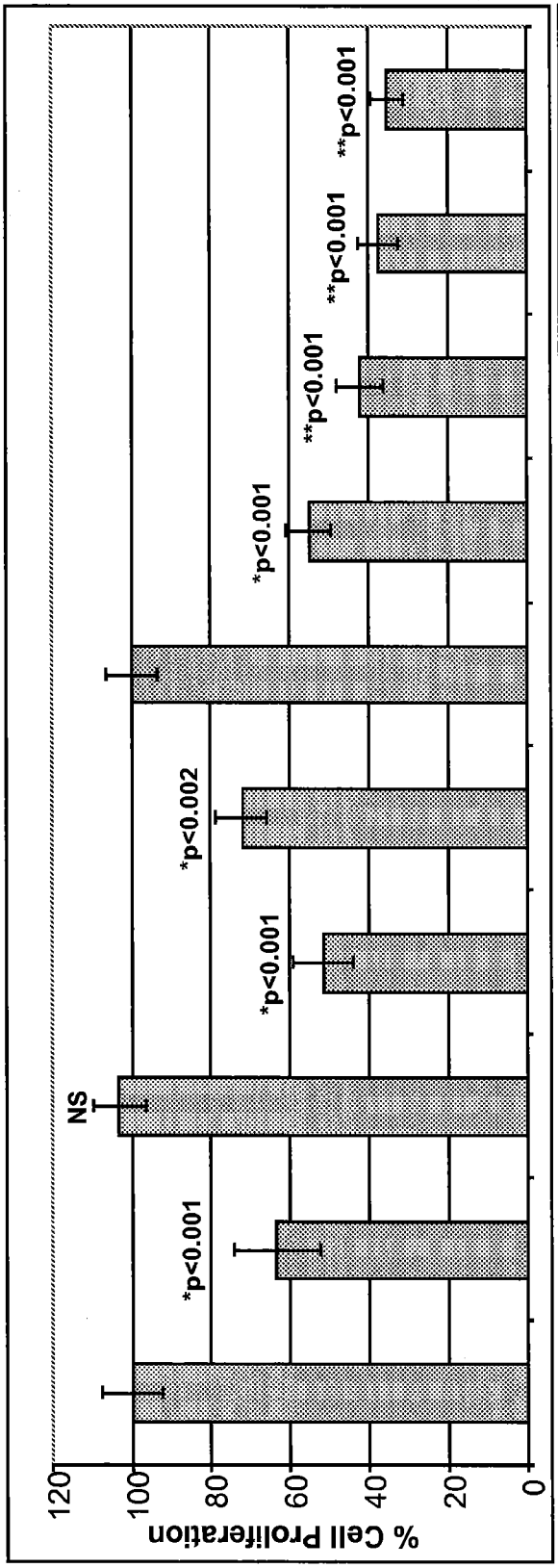
Figure 8



IgG	10***							20	10	10	10	+
DMSO		+							+			
U1-59			10							10	10	10
c2C4				10							10	
trastuzumab					10							10
lapatinib						+						

* = versus control treatment
** = versus either single agent alone
NS = not significant
*** = μg/ml

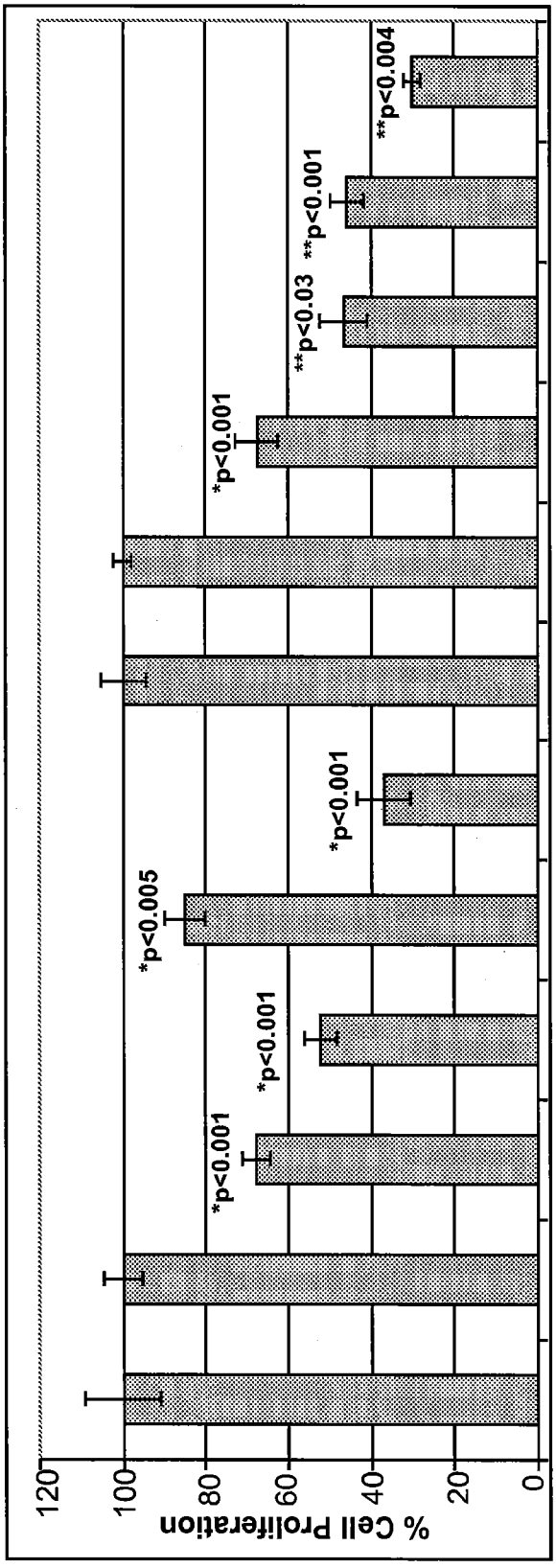
Figure 9



IgG	10***					20	10				10
U1-59		10					10	10			10
cetuximab			10				10		10		
c2C4				10						10	
trastuzumab					10						10

* = versus control treatment
** = versus either single agent alone
NS = not significant
*** = μg/ml

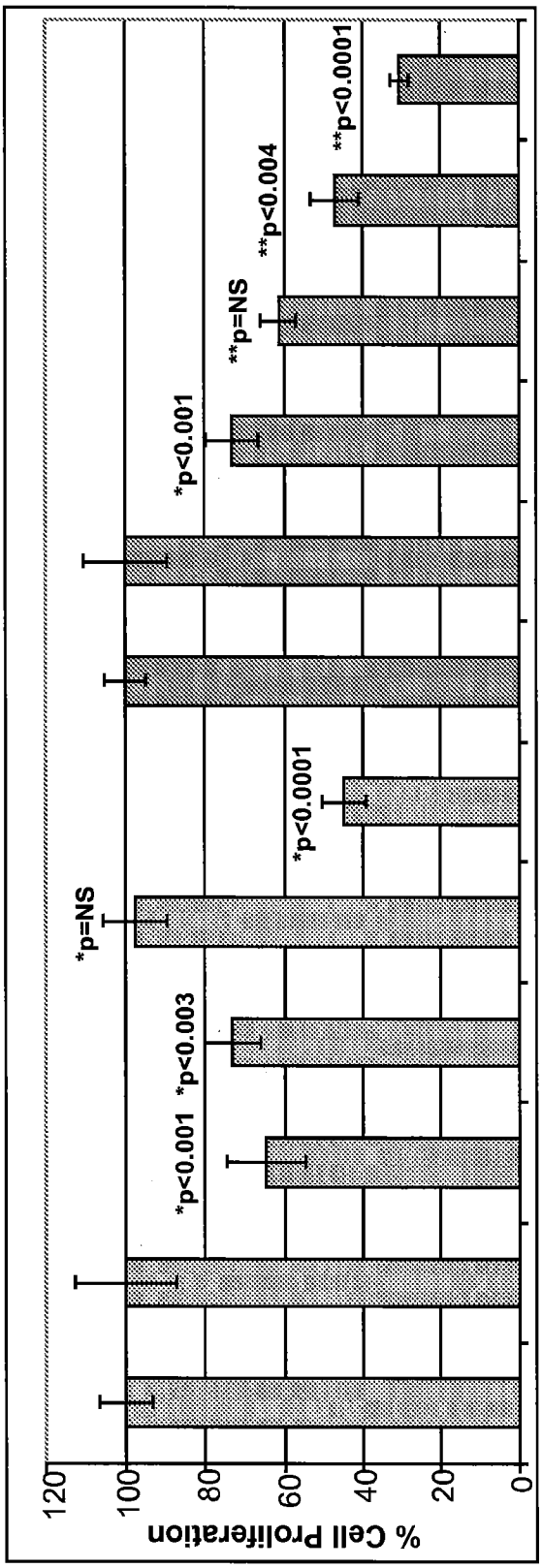
Figure 10



IgG	10***					20	10	10			
DMSO		+					+	10	10		
U1-59			10					10	10	10	10
c2C4				10					10		
trastuzumab					10					10	
Lapatinib (1.5 µM in DMSO)						+					+

* = versus control treatment
** = versus either single agent alone
NS = not significant
*** = µg/ml

Figure 11



IgG	10***						10	20	10		10	
DMSO		+					+					
U1-59			10						10	10	10	
c2C4				10						10		
trastuzumab					10							
Lapatinib (1 µM in DMSO)						+						+

* = versus control treatment
** = versus either single agent alone
NS = not significant
*** = µg/ml

Figure 12

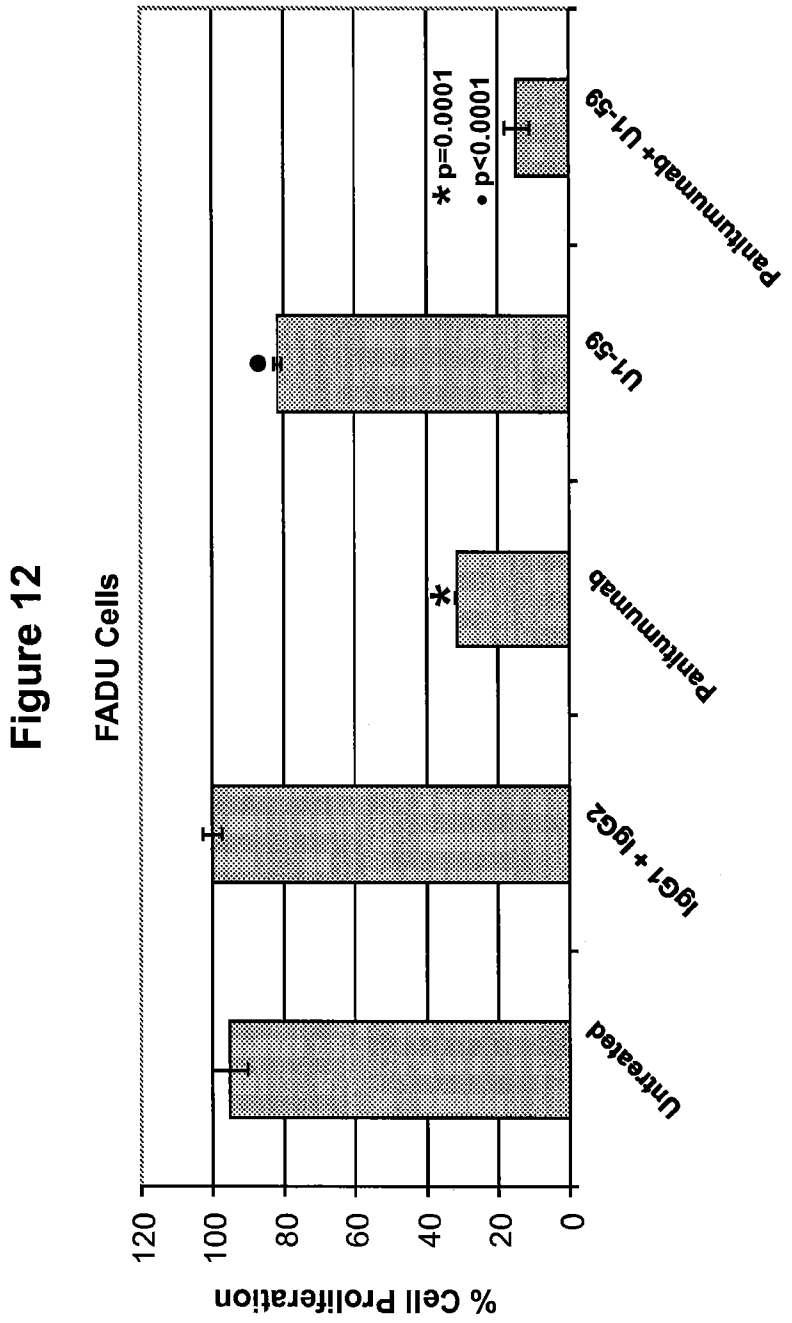

FADU Cells
% Cell Proliferation
120
100
80
60
40
20
0
* p=0.0001
• p<0.0001
Untreated
IgG1 + IgG2
Panitumumab
U1-59
Panitumumab+ U1-59

Figure 13

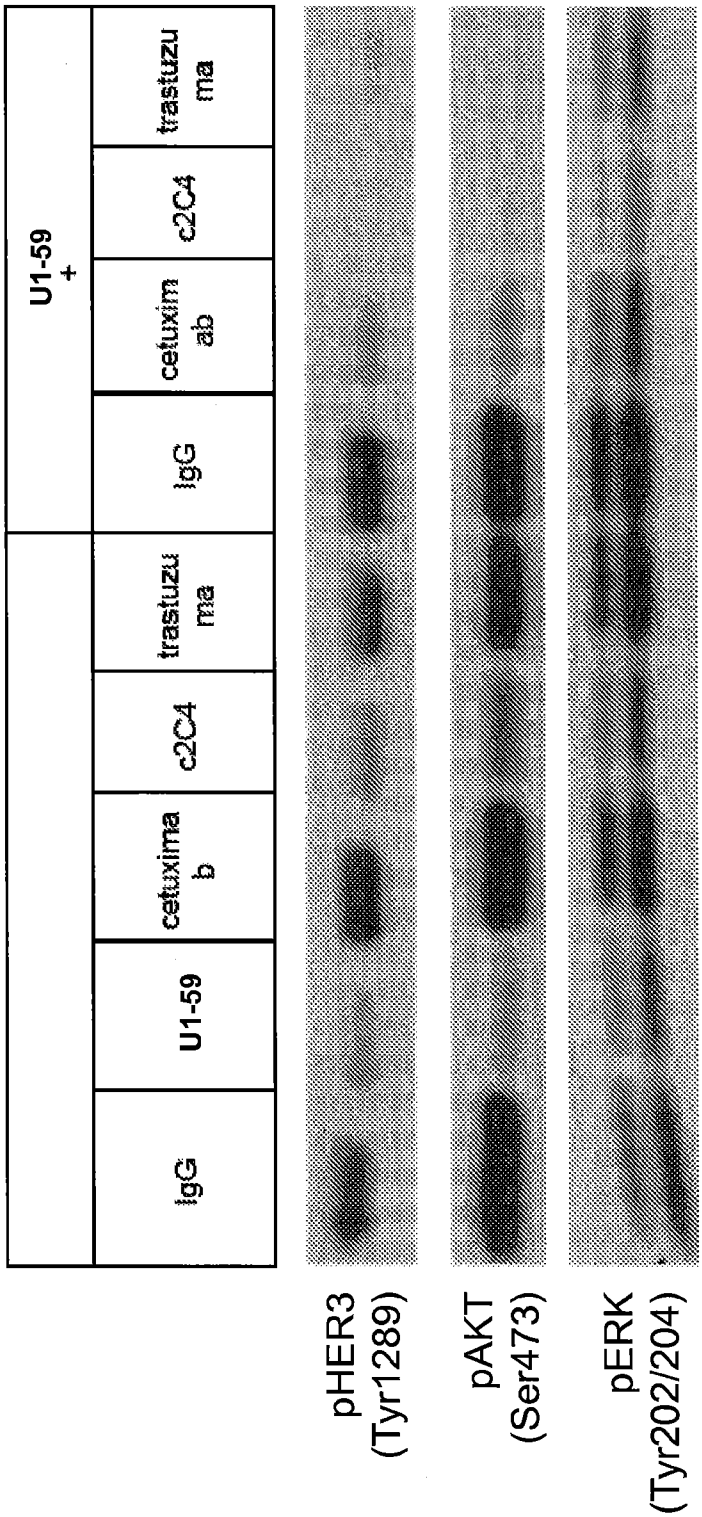

IgG
U1-59
cetuximab
c2C4
trastuzumab
U1-59 +
IgG
cetuximab
c2C4
trastuzumab
pHER3 (Tyr1289)
pAKT (Ser473)
pERK (Tyr202/204)

Figure 14

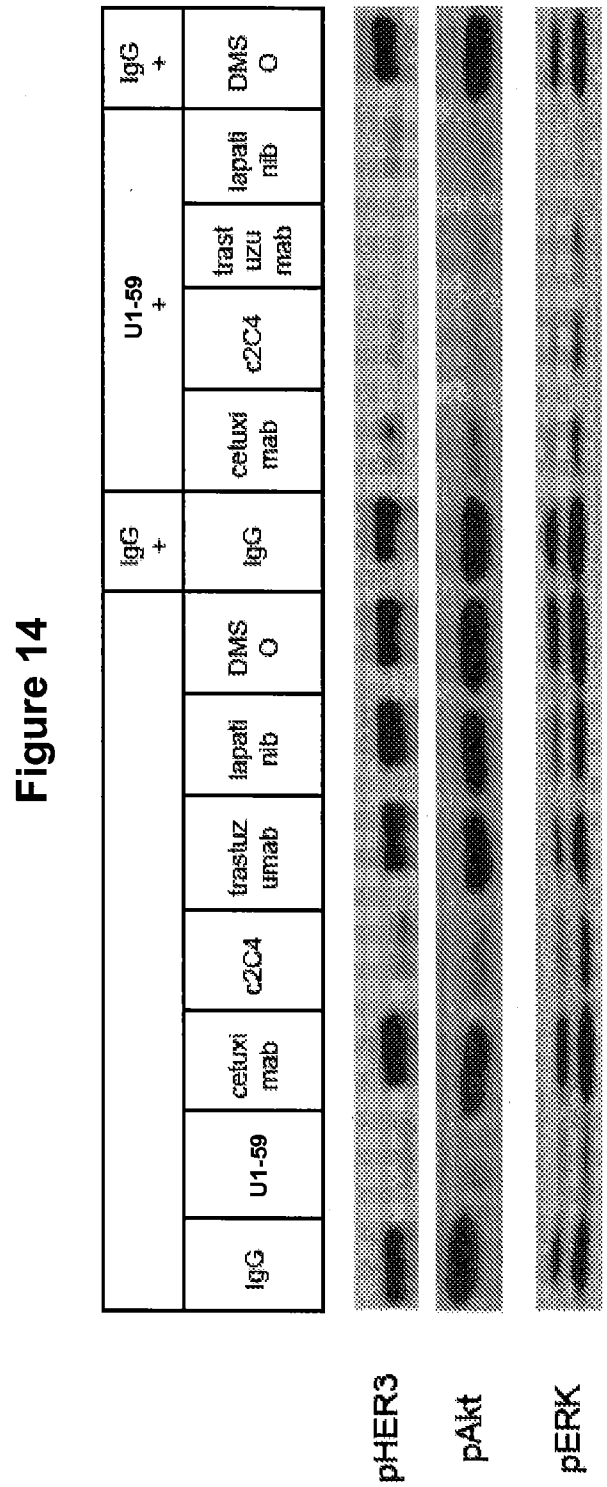

IgG
U1-59
cetuximab
c2C4
trastuzumab
lapatinib
DMSO
IgG + IgG
U1-59 + cetuximab
U1-59 + c2C4
U1-59 + trastuzumab
U1-59 + lapatinib
IgG + DMSO
pHER3
pAkt
pERK

Figure 15

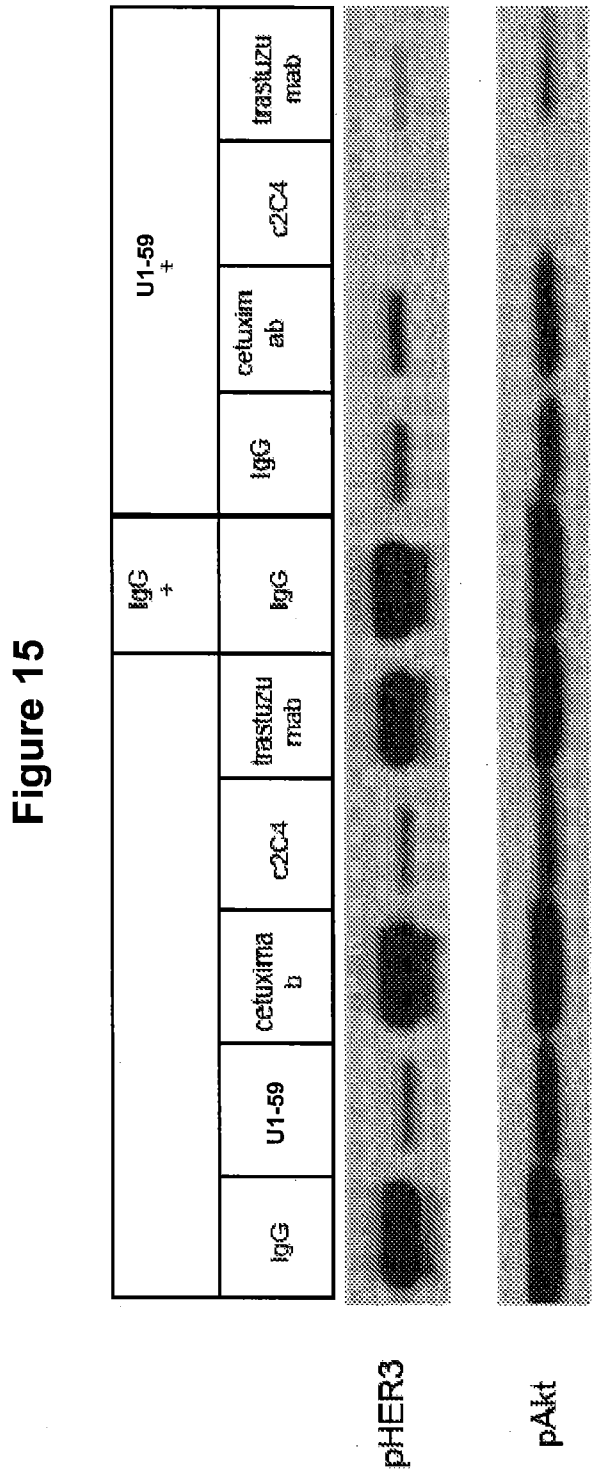

IgG
U1-59
cetuximab
c2C4
trastuzumab
IgG ‡
U1-59 ‡
IgG
cetuximab
c2C4
trastuzumab
pHER3
pAkt

Figure 16

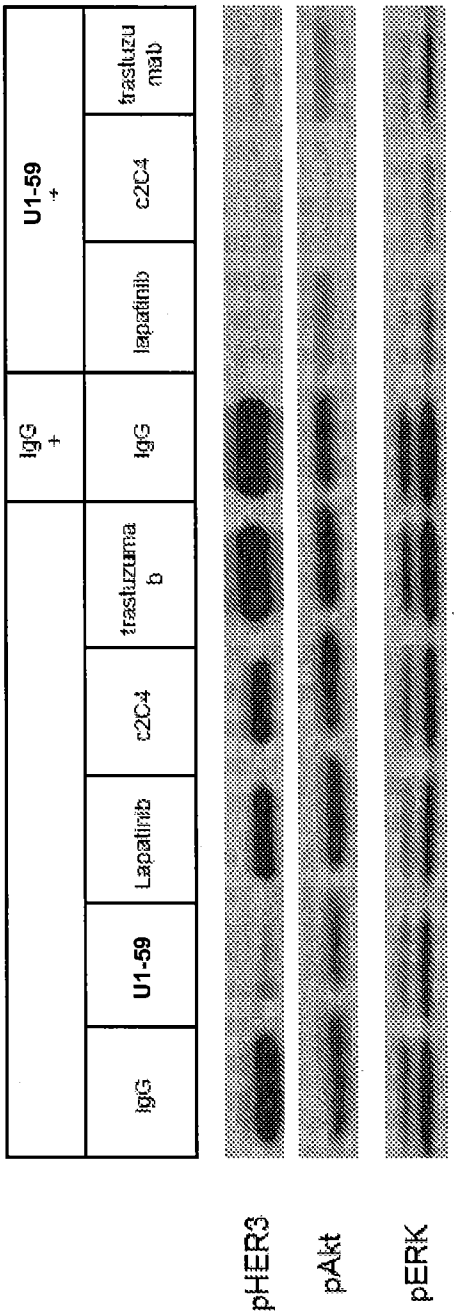

IgG
U1-59
Lapatinib
c2C4
trastuzuma b
IgG +
U1-59 +
lapatinib
c2C4
trastuzu mab
pHER3
pAkt
pERK

Figure 17

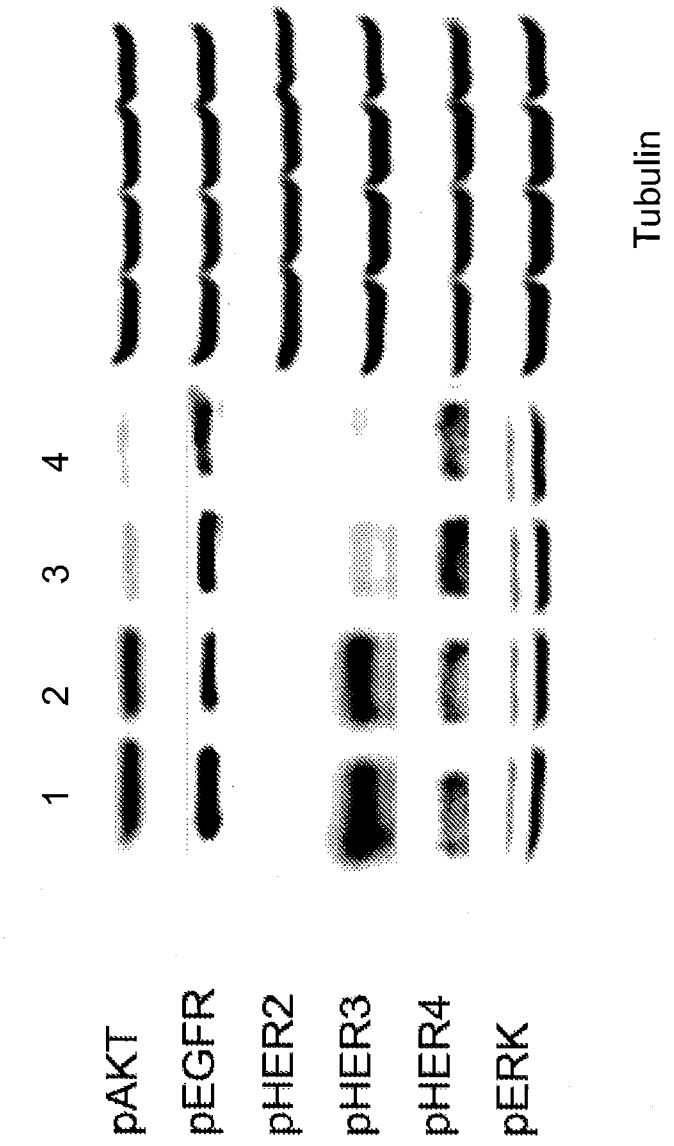


Figure 18

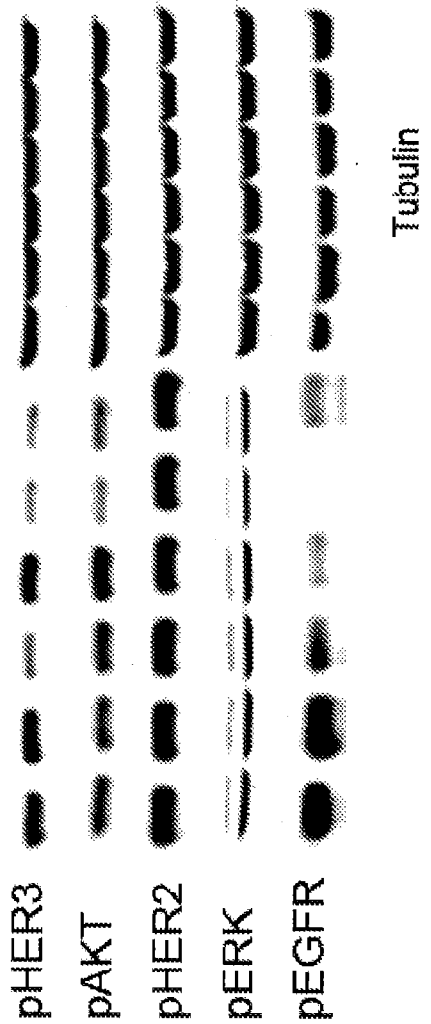


Figure 19

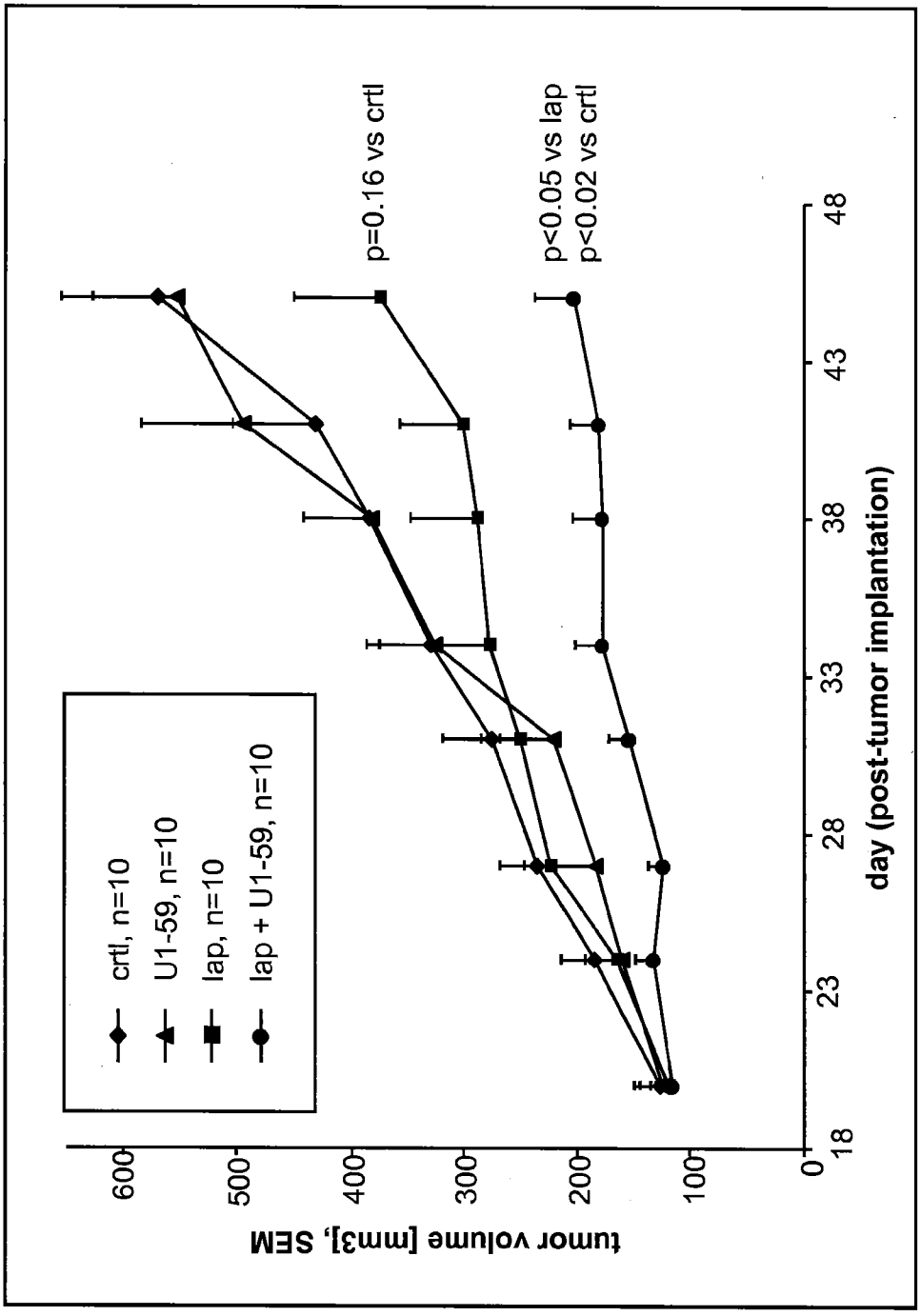
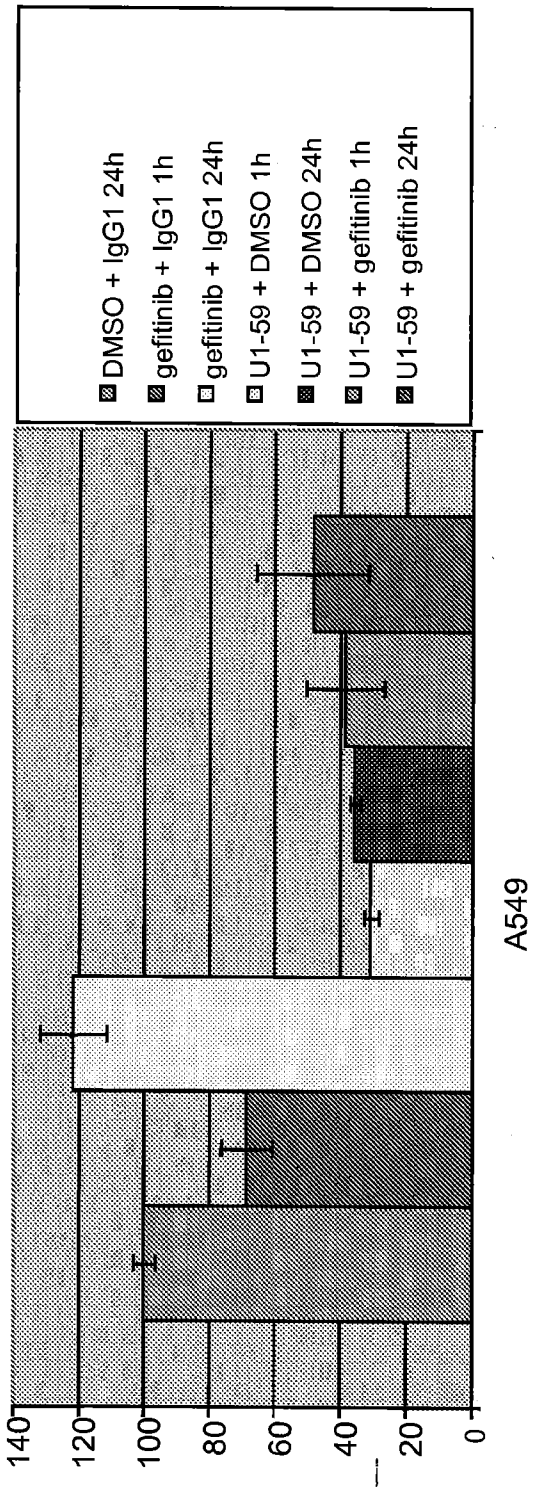

tumor volume [mm3], SEM
600
500
400
300
200
100
0
18
23
28
33
38
43
48
day (post-tumor implantation)
crtl, n=10
U1-59, n=10
lap, n=10
lap + U1-59, n=10
p=0.16 vs crtl
p<0.05 vs lap
p<0.02 vs crtl

Figure 20


DMSO + IgG1 24h
gefitinib + IgG1 1h
gefitinib + IgG1 24h
U1-59 + DMSO 1h
U1-59 + DMSO 24h
U1-59 + gefitinib 1h
U1-59 + gefitinib 24h
140
120
100
80
60
40
20
0
A549

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO 2007077028 A **[0004]**
- US 4816397 A **[0030] [0031]**
- US 5916771 A **[0030] [0031]**
- US 6207418 B **[0030] [0031]**
- US 4816567 A **[0042]**
- US 20030217373 A **[0046]**
- US 5939598 A **[0046]**
- US 6075181 A **[0046]**
- US 6114598 A **[0046]**
- US 6150584 A **[0046]**
- US 6162963 A **[0046]**
- US 6673986 B **[0046]**
- US 6833268 B **[0046]**
- US 7435871 B **[0046]**
- JP 3068180 B **[0046]**
- JP 3068506 B **[0046]**
- JP 3068507 B **[0046]**
- EP 0463151 A **[0046]**
- WO 9402602 A **[0046]**
- WO 9634096 A **[0046]**
- WO 9824893 A **[0046] [0050]**
- WO 0076310 A **[0046] [0050]**
- US 5545806 A **[0047]**
- US 5545807 A **[0047]**
- US 5569825 A **[0047]**
- US 5591669 A **[0047]**
- US 5612205 A **[0047]**
- US 5625126 A **[0047]**
- US 5625825 A **[0047]**
- US 5633425 A **[0047]**
- US 5643763 A **[0047]**
- US 5661016 A **[0047]**
- US 5721367 A **[0047]**
- US 5770429 A **[0047]**
- US 5789215 A **[0047]**
- US 5789650 A **[0047]**
- US 5814318 A **[0047]**
- US 5874299 A **[0047]**
- US 5877397 A **[0047]**
- US 5981175 A **[0047]**
- US 6023010 A **[0047]**
- US 6255458 B **[0047]**
- EP 0546073 A **[0047]**
- WO 9203918 A **[0047]**
- WO 9222645 A **[0047]**
- WO 9222647 A **[0047]**
- WO 9222670 A **[0047]**
- WO 9312227 A **[0047]**
- WO 9400569 A **[0047]**
- WO 9425585 A **[0047]**
- WO 9614436 A **[0047]**
- WO 9713852 A **[0047]**
- WO 9824884 A **[0047]**
- EP 773288 A **[0048]**
- EP 843961 A **[0048]**
- US 75962096 A **[0050]**
- US 4399216 A **[0056]**
- US 4912040 A **[0056]**
- US 4740461 A **[0056]**
- US 4959455 A **[0056]**
- US 20030104625 A **[0061]**
- US 6204388 B **[0061]**
- US 6303342 B **[0061]**
- WO 9526961 A **[0061]**
- WO 961259 A **[0061]**
- US 7557182 B **[0061]**
- EP 639577 A **[0061]**
- US 4939168 A **[0061]**
- US 5681847 A **[0061]**
- US 4046878 A **[0062]**
- US 4471052 A **[0062]**
- EP 137145 A **[0067]**
- EP 56692 A **[0067]**
- WO 0503707 A **[0101]**
- WO 04091384 A **[0101]**
- WO 04011900 A **[0101]**
- WO 03013602 A **[0107]**
- US 20080124345 A **[0107] [0151] [0152] [0154] [0155] [0156] [0157] [0158] [0159] [0161] [0162] [0164] [0167] [0170] [0174] [0178] [0182] [0185]**
- WO 03048730 A2 **[0124]**

Non-patent literature cited in the description

- **PLOWMAN et al.** *Proc. Natl. Acad. Sci. US,* 1990, vol. 87, 4905-4909 **[0002]**
- **KRAUS et al.** *Proc. Natl. Acad. Sci. US,* 1989, vol. 86, 9193-9197 **[0002]**
- **KRAUS et al.** *Proc. Natl. Acad. Sci. US,* 1993, vol. 90, 2900-2904 **[0002]**

- **SAMBROOK et al.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 2001 **[0019]**
- **AUSUBEL et al.** Current Protocols in Molecular Biology. Greene Publishing Associates, 1992 **[0019]**
- **HARLOW ; LANE.** Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1990 **[0019]**
- **BINZ ; PLÜCKTHUN.** *Curr. Opin. Biotechnol.,* 2005, vol. 16, 459-69 **[0025]**
- **SKERRA.** *J. Mol. Recog.,* 2000, vol. 13, 167-87 **[0025]**
- **JONES et al.** *Nature,* 1986, vol. 321, 522-525 **[0026]**
- **RIECHMANN et al.** *Nature,* 1988, vol. 332, 323-329 **[0026]**
- **PRESTA.** *Curr. Op. Struct. Biol.,* 1992, vol. 2, 593-596 **[0026]**
- **MORRISON et al.** *Proc. Natl. Acad. Sci. US,* 1984, vol. 81, 6851-6855 **[0026]**
- **HOLLINGER et al.** *Proc. Natl. Acad. Sci. US,* 1993, vol. 90, 6444-6448 **[0026]**
- **PLÜCKTHUN.** The Pharmacology of Monoclonal Antibodies. Springer Verlag, 1994, vol. 113, 269-315 **[0026]**
- **MUYLDERMANS et al.** *Rev. Mol. Biotechnol.,* 2001, vol. 74, 277-302 **[0027]**
- **HOLT et al.** *Trends Biotechnol.,* 2003, vol. 21, 484-90 **[0027]**
- **IDUSOGIE et al.** *J. Immunol.,* 2001, vol. 166, 2571-2575 **[0030]**
- **SHIELDS et al.** *J. Biol. Chem.,* 2001, vol. 276, 6591-604 **[0031]**
- Immunology - A Synthesis. Sinauer Associates, 1991 **[0036]**
- **BOWIE et al.** *Science,* 1991, vol. 253, 164 **[0037]**
- Proteins, Structures and Molecular Principles. W H. Freeman and Company, 1984 **[0037]**
- Introduction to Protein Structure. Garland Publishing, 1991 **[0037]**
- **THORNTON et al.** *Nature,* 1991, vol. 354, 105 **[0037]**
- **KÖHLER et al.** *Nature,* 1975, vol. 256, 495 **[0041]**
- **CLACKSON et al.** *Nature,* 1991, vol. 352, 624-628 **[0043]**
- **MARKS et al.** *J. Mol. Biol.,* 1991, vol. 222, 581-597 **[0043]**
- **FELDHAUS ; SIEGEL.** *J. Immunol. Methods,* 2004, vol. 290, 69-80 **[0043]**
- **GROVES ; OSBOURN.** *Expert Opin. Biol. Ther.,* 2005, vol. 5, 125-135 **[0043]**
- **JOSTOCK ; DUBEL.** *Comb. Chem. High Throughput Screen,* 2005, vol. 8, 127-133 **[0043]**
- **MENDEZ et al.** *Nature Genetics,* 1997, vol. 15, 146-156 **[0045] [0050]**
- **GREEN AND JAKOBOVITS.** *J. Exp. Med.,* 1998, vol. 188, 483-495 **[0045]**
- The McGraw-Hill Dictionary of Chemical Terms. McGraw-Hill, 1985 **[0073]**
- **HAZRA et al.** *Cell Biophys,* 1994, vol. 24-25, 1-7 **[0077]**
- **WITZIG.** *Cancer Chemother. Pharmacol.,* 2001, vol. 48 (1), 91-95 **[0077]**
- **HAMANN et al.** *Bioconjug. Chem.,* 2002, vol. 13, 40-46 **[0078]**
- **MANDLER et al.** *J. Natl. Cancer Inst.,* 2000, vol. 92, 1549-1551 **[0078]**
- **LIU et al.** *Proc. Natl. Acad. Sci. US,* 1996, vol. 93, 8618-8623 **[0078]**
- **OTANI et al.** *Jpn. J. Cancer Res.,* 2000, vol. 91, 837-44 **[0079]**
- **LI et al.** *Int. J. Mol. Med.,* 1999, vol. 3, 647-53 **[0079]**
- Remington's Pharmaceutical Sciences. Mack Publishing Company, 1990 **[0082]**
- **BALDRICK.** *Regul. Toxicol. Pharmacol.,* 2000, vol. 32, 210-218 **[0082]**
- **WANG.** *Int. J. Pharm.,* 2000, vol. 203, 1-60 **[0082]**
- **CHARMAN.** *J. Pharm. Sci.,* 2000, vol. 89, 967-978 **[0082]**
- **POWELL et al.** *PDA J. Pharm. Sci. Technol.,* 1998, vol. 52, 238-311 **[0082]**
- **PRICE et al.** *Methods Mol. Biol.,* 2002, vol. 218, 255-268 **[0101]**
- **WALLASCH et al.** *EMBO J.,* 1995, vol. 14, 4267-4275 **[0104]**
- **KEARNEY et al.** *J. Immunol.,* 1979, vol. 123, 1548-1550 **[0113]**
- **JIA et al.** *J Immunol Methods.,* 2004, vol. 288, 91-98 **[0137]**
- **CHOTHIA et al.** *J. Mol. Biol.,* 1987, vol. 196, 901-17 **[0143]**
- **CHOTHIA et al.** *Nature,* 1989, vol. 342, 877-83 **[0144]**
- **MARTIN et al.** *J. Mol. Biol.,* 1996, vol. 263, 800-15 **[0144]**
- **BOVEN et al.** *Cancer Research,* 1992 **[0162]**
- **SIMEONI et al.** *Cancer Res.,* 2004, vol. 64, 1094-1101 **[0213]**

Anyag és eljárások HER-3-mal összefüggő betegségek kezelésére vagy megelőzésére

Szabadalmi igénypontok

1. Egy első hatóanyag és egy második hatóanyag HER-3-mal összefüggő hiperproliferatív betegség kezelésére vagy megelőzésére történő alkalmazásra,
ahol az említett hiperproliferatív betegség egy olyan rákbetegség, amelyre a HER-3 expressziója vagy túlzott mértékű expressziója jellemző,
ahol az említett második hatóanyag a trastuzumab, és

ahol az említett első hatóanyag egy HER-3-hoz kötődő antigénkötő protein, és tartalmaz. egy nehéz lánc aminosav-szekvenciát, amely tartalmaz egy SEQ ID NO: 256 szekvenciaként bemutatott CDRH1-et; egy SEQ ID NO: 282 szekvenciaként bemutatott CDRH2-t; és egy SEQ ID NO: 315 szekvenciaként bemutatott CDRH3-at; és tartalmaz egy könnyű lánc aminosav-szekvenciát, amely tartalmaz egy SEQ ID NO: 340 szekvenciaként bemutatott CDRL1-et; egy SEQ ID NO: 344 szekvenciaként bemutatott CDRL2-t; és egy SEQ ID NO: 387 szekvenciaként bemutatott CDRL3-at.

2. Az 1. igénypont szerinti első hatóanyag és második hatóanyag az igényelt alkalmazásra, ahol az említett első hatóanyag egy HER-3-hoz kötődő antigénkötő protein, és tartalmaz egy SEQ ID NO: 70 szekvenciaként bemutatott nehéz lánc aminosav-szekvenciát és/vagy egy SEQ ID NO: 72 szekvenciaként bemutatott könnyű lánc aminosav-szekvenciát.

3. Az 1. igénypont szerinti első hatóanyag és második hatóanyag az igényelt alkalmazásra, ahol az említett első hatóanyag egy HER-3-hoz kötődő antigénkötö protein, és tartalmazza a SEQ ID NO: 70 szekvenciaként bemutatott nehéz lánc aminosav-szekvenciát és a SEQ ID NO: 72 szekvenciaként bemutatott könnyű lánc aminosav-szekvenciát.

4. A 3-6. igénypontok bármelyike szerinti első hatóanyag és második hatóanyag az igényelt alkalmazásra, ahol az említett antigénkötő protein a HER-3 extracelluláris doménje ellen irányul, vagy csökkenti a HER-3-közvetítette szignáltranszdukciót, vagy csökkenti a HER-3-foszforilációt, vagy csökkenti a sejtproliferációt vagy csökkenti a sejtmigrációt és/vagy fokozza a HER-3 csökkentö szabályozását („downregulation").

5. Az előző igénypontok bármelyike szerinti első hatóanyag és második hatóanyag az

igényelt alkalmazásra, ahol az említett, HER-3-hoz kötődő antigénkötő protein egy antitest, különösen monoklonális antitest, poliklonális antitest, rekombináns antitest, humanizált antitest, humán antitest, kimérikus antitest, multispecifikus antitest vagy ezek antitest-fragmense, különösen egy Fab-fragmens, Fab'-fragmens, $F(ab')_2$-fragmens, Fv-fragmens, egy diatest vagy egyláncú antitest-molekula, különösen IgG1, IgG2, IgG3 vagy IgG4 típusba tartozó antitest.

6. Az 1. igénypont szerinti első hatóanyag és második hatóanyag az igényelt alkalmazásra, ahol az említett első hatóanyag egy effektor-csoporthoz, különösen egy radioaktív izotóphoz vagy radionuklidhoz, toxinhoz vagy egy terápiás vagy kemoterápiás csoporthoz van kapcsolva, ahol a terápiás vagy kemoterápiás csoport előnyösen calicheamicin, auristatinPE, geldanamicin, maytansin és ezek származékai.

7. Az 1. igénypont szerinti első hatóanyag és második hatóanyag az igényelt alkalmazásra, ahol az említett kezelés vagy megelőzés magában foglalja egy további terápiás hatóanyag adagolását és/vagy sugárterápia alkalmazását, ahol a további terápiás hatóanyag előnyösen egy antineoplaziás hatóanyag vagy egy tumorellenes antitest vagy egy kemoterápiás hatóanyag, ahol a kemoterápiás hatóanyag előnyösen a következőkből álló csoportból van kiválasztva: capecitabin, anthracyclin, doxorubicin, ciklofoszfamid, paclitaxel, docetaxel, cisplatin, gemcitabin és carboplatin.

8. Az 1. igénypont szerinti első hatóanyag és második hatóanyag az igényelt alkalmazásra, ahol az említett első hatóanyag és az említett második hatóanyag intravénásan, szubkután, intramuszkulárisan vagy orálisan adagolható.

9. Az 1. igénypont szerinti első hatóanyag és második hatóanyag az igényelt alkalmazásra, ahol az említett betegség a következőkből álló csoportból van kiválasztva: emlőrák, petefészekrák, prosztatarák, vastagbélrák, veserák, tüdőrák, hasnyálmirigyrák, epidermoid karcinóma, fibroszarkóma, melanóma, nasopharyngealis karcinóma és pikkelysejtes karcinóma.

10. Az 1. igénypont szerinti első hatóanyag és második hatóanyag az igényelt alkalmazásra, ahol az említett kezelés vagy megelőzés magában foglalja az említett első hatóanyag vagy második hatóanyag körülbelül 1 mg/testtömeg-kilogramm és körülbelül 20 mg/testtömeg-kilogramm közötti dózisban, legalább hat hetenként egyszeri adagolását.

11. Az 1. igénypont szerinti első hatóanyag és második hatóanyag az igényelt alkal-

mazásra, ahol az említett kezelés vagy megelőzés a betegnek történő adagolást követően magában foglalja a terápiás eredmény ellenőrzését is.