



(51) International Patent Classification:

A61K 39/395 (2006.01) *C07K 16/32* (2006.01)
A61K 31/337 (2006.01) *A61P 35/00* (2006.01)
A61K 31/565 (2006.01)

(21) International Application Number:

PCT/EP2016/080890

(22) International Filing Date:

14 December 2016 (14.12.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

15200776.1 17 December 2015 (17.12.2015) EP

(71) Applicant (for all designated States except US): **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, 4070 Basel (CH).

(71) Applicant (for US only): **HOFFMANN-LA ROCHE INC.** [US/US]; Great Notch, 150 Clove Road, 8th Floor, Little Falls, New Jersey 07424 (US).

(72) Inventors: **BOSSENMAIER, Birgit**; Horst-Wolfram-Geissler-Weg 10, 82229 Seefeld (DE). **CEPPI, Maurizio**; Heilmannstrasse 9, 81479 Muenchen (DE). **HASMAN, Max**; Rudolf-Zenker-Str. 10, 81377 Muenchen (DE). **WEISSER, Martin**; Primelstr. 1, 82377 Penzberg (DE).

(74) Agents: **JENNI, Wolfgang** et al.; Roche Diagnostics GmbH, Patent Department (LPP.....6164), P.O. Box 11 52, 82372 Penzberg (DE).

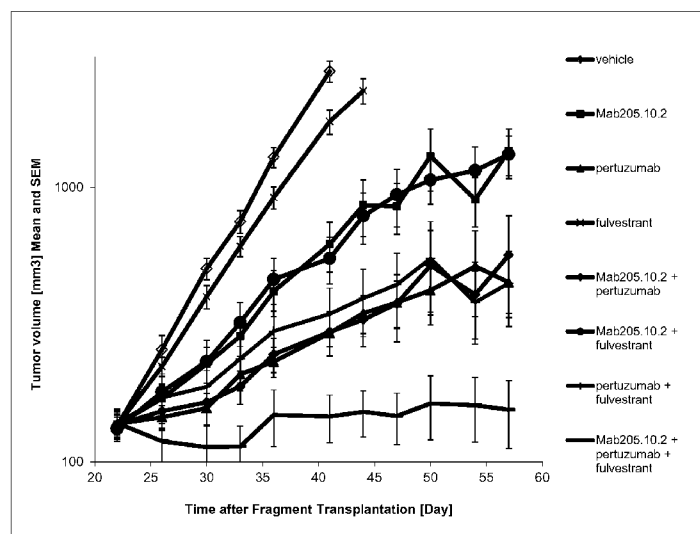
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS,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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: COMBINATION THERAPY OF ANTI-HER3 ANTIBODIES AND ANTI-HER2 ANTIBODIES

Fig. 6

(57) Abstract: The present disclosure relates to the combination therapy of anti-HER3 antibodies with certain anti-HER2 antibodies.

Combination therapy of anti-HER3 antibodies and anti-HER2 antibodies

The present invention relates to the combination therapy of anti-HER3 antibodies and anti-HER2 antibodies, in patients suffering from a HER2 low, HER3 positive and hormone receptor (HR) positive breast cancer including dose schedules and additional therapies.

5 Background of the Invention

Human HER3 (ErbB-3, ERBB3, c-erbB-3, c-erbB3, receptor tyrosine-protein kinase erbB-3, SEQ ID NO: 17) encodes a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases which also includes HER1 (also known as EGFR), HER2, and HER4 (Kraus, M.H. et al, PNAS 86 (1989) 9193-9197; Plowman, G.D. et al, PNAS 87 (1990) 4905-4909; Kraus, M.H. et al, PNAS 90 (1993) 2900-2904). Like the prototypical epidermal growth factor receptor, the transmembrane receptor HER3 consists of an extracellular ligand-binding domain (ECD), a dimerization domain within the ECD, a transmembrane domain, an intracellular protein tyrosine kinase domain (TKD) and a C-terminal phosphorylation domain. HER3 has a heregulin (HRG) binding domain within the extracellular domain but its kinase domain is inactive. Therefore, when activated by binding of its ligand, HER3 can form heterodimers with other HER family members, but cannot transphosphorylate the partner protein. However, heterodimerization of HER3 leads to the activation of the receptor-mediated signaling pathway through transphosphorylation of its intracellular domain by the dimerization partner. Dimer formation between HER family members expands the signaling potential of HER3 and is a means not only for signal diversification but also signal amplification. For example the HER2/HER3 heterodimer induces one of the most important mitogenic signals via the PI3K and AKT pathway among HER family members (Sliwkowski M.X., et al, J. Biol. Chem. 269 (1994) 14661-14665; Alimandi M, et al, Oncogene. 10 (1995) 1813-1821; Hellyer, N.J., J. Biol. Chem. 276 (2001) 42153-4261; Singer, E., J. Biol. Chem. 276 (2001) 44266-44274; Schaefer, K.L., Neoplasia 8 (2006) 613-622).

Expression of this gene and/or expression of its protein have been reported in numerous cancers, including prostate, bladder, and breast tumors. Alternate transcriptional splice variants encoding different isoforms have been characterized. One isoform lacks the intermembrane region and is secreted outside the cell. This

form acts to modulate the activity of the membrane-bound form. Additional splice variants have also been reported, but they have not been thoroughly characterized.

WO 97/35885 relates to HER3 antibodies. WO 2003/013602 relates to inhibitors of HER activity, including HER antibodies. WO 2007/077028, WO 2008/100624 ,
5 WO2011076683, WO2011044311, WO2011136911, WO2012019024,
WO2012022814, WO2012031198, WO2012044612, WO2012052230,
WO2012059858 relate to HER3 antibodies.

Human HER2 refers to 185-kDa growth factor receptor also referred to as neu and c-erbB-2 (Slamon, et al., Science 235 (1987) 177-182; Swiss-Prot P04626) whose
10 function is related to neoplastic transformation in human breast cancer cells. Overexpression of this protein has been identified in 20-30% of breast cancer patients where it correlates with regionally advanced disease, increased probability of tumor recurrence, and reduced patient survival. As many as 30-40% of patients having gastric, endometrial, salivary gland, non-small cell lung, pancreatic,
15 ovarian, peritoneal, prostate, or colorectal cancers may also exhibit overexpression of this protein.

The HER receptor will generally comprise an extracellular domain, which may bind an HER ligand; a lipophilic transmembrane domain, a conserved intracellular tyrosine kinase domain, and a carboxyl-terminal signaling domain harboring
20 several tyrosine residues which can be phosphorylated. The extracellular domain of HER2 comprises four domains, Domain I (amino acid residues from about 1-195), Domain II (amino acid residues from about 196-320), Domain III (amino acid residues from about 321-488), and Domain IV (amino acid residues from about 489-632) (residue numbering without signal peptide). See Garrett, et al., Mol. Cell.
25 11 (2003) 495-505, Cho, et al., Nature 421 (2003) 756-760, Franklin, et al., Cancer Cell 5 (2004) 317-328, or Plowman, et al., Proc. Natl. Acad. Sci. 90 (1993) 1746-1750 and WO 2006/007398.

Trastuzumab (e.g. Herceptin®) is a recombinant humanized anti-HER2 monoclonal antibody used for the treatment of HER2 over-expressed/HER2 gene
30 amplified metastatic breast cancer. Trastuzumab binds specifically to the same epitope of HER2 as the murine anti-HER2 antibody 4D5 described in Hudziak, et al., Mol. Cell. Biol. 9 (1989) 1165-1172. Trastuzumab is a recombinant humanized version of the murine anti-HER2 antibody 4D5, referred to as rhuMAb 4D5 or trastuzumab) and has been clinically active in patients with HER2-overexpressing

metastatic breast cancers that had received extensive prior anticancer therapy. (Baselga, et al, J. Clin. Oncol. 14 (1996) 737-744). Trastuzumab and its method of preparation are described in US 5,821,337.

5 Pertuzumab (e.g. Perjeta®) is another recombinant humanized anti-HER2 monoclonal antibody used for the treatment of HER2 positive cancers. Pertuzumab binds specifically to sub-domain II, a different epitope on the extracellular domain of HER2 than trastuzumab's binding site on sub-domain IV. Pertuzumab is the first in a new class of HER2 dimerization inhibitors (HDIs). Through its binding to the HER2 extracellular domain, pertuzumab inhibits dimerization of HER2 (with other
10 HER family members), thereby inhibiting downstream signaling pathways and cellular processes associated with tumor growth and progression (Franklin, M.C., et al. Cancer Cell 5 (2004) 317-328 and Friess, T, et al. Clin Cancer Res 11 (2005) 5300-5309). Pertuzumab is a recombinant humanized version of the murine anti-HER2 antibody 2C4 (referred to as rhuMAb 2C4 or pertuzumab) and it is described
15 together with the respective method of preparation in WO 01/00245 and WO 2006/007398.

The "epitope 2C4" is the region in the extracellular domain of HER2 to which the antibody 2C4 binds. In order to screen for antibodies which bind to the 2C4 epitope, a routine cross-blocking assay such as that described in "Ed. Harlow and
20 David Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)", can be performed. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 2C4 epitope of HER2 (e.g. any one or more residues in the region from about residue 22 to about residue 584 of HER2, inclusive). Epitope 2C4 comprises residues from domain II in the extracellular
25 domain of HER2. 2C4 and pertuzumab bind to sub-domain II of the extracellular domain of HER2 near the junction of domains I, II and III. See also Franklin, et al., Cancer Cell 5 (2004) 317-328.

Summary of the Invention

30 The invention relates to an antibody which binds to human HER3 for use in the treatment of breast cancer in combination with an antibody binding to human HER2 and inhibiting dimerization of HER2, wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive.

In one preferred embodiment the breast cancer is HER2 low, HER3 positive and ER (estrogen receptor) positive.

In one embodiment additionally either

A) an anti-hormonal agent is co-administered , wherein the anti-hormonal agent is selected from the group of:

a) a selective estrogen receptor degrader (SERD), like e.g. fulvestrant,
5 or

b) a selective estrogen receptor modulator (SERM); and such as
tamoxifen, raloxifene, toremifene or lasofoxifene, or

c) an aromatase inhibitor; (in one preferred embodiment the
aromatase inhibitor is letrozole, anastrozole or exemestane;
10 or

B) a chemotherapeutic agent is co-administered , wherein the chemotherapeutic agent is selected from the group of:

a) a taxane or taxane derivative

b) cyclophosphamide,

c) cisplatin or carboplatin
15

d) mitomycin C,

e) methotrexate,

f) 5-fluorouracil or capecitabine,

g) doxorubicin or its liposomal formulation, or daunorubicin or its
liposomal formulation,
20

h) gemcitabine,

i) mitoxantrone,

j) vinorelbine,

k) eribulin, and

l) ixabepilone.
25

In one embodiment the combination is administered as a first-line treatment.

5 In one embodiment the antibody which binds to human HER3 is characterized in that the heavy chain variable domain comprises a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6 or a CDR1L region of SEQ ID NO:7.

10 In one embodiment the antibody which binds to human HER3 is characterized in comprising as heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.

15 In one embodiment the antibody which binds to human HER3 is characterized in that

the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:10.

20 In one embodiment the antibody which binds to human HER3 described above is further characterized in that the antibody is of IgG1 subclass and is glycosylated with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65 % or lower.

In one embodiment the antibody binding to human HER2 and inhibiting dimerization of HER2 is pertuzumab.

25 In one embodiment the antibody which binds to human HER3 is administered at a dose of 400-600 mg every 3 weeks and the antibody which binds to human HER2 is administered at a dose of 300-500 mg every 3 weeks.

30 Another aspect of the invention is an antibody binding to human HER2 and inhibiting dimerization of HER2 for use in the treatment of breast cancer in combination with an antibody which binds to human HER3, wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive. In one preferred embodiment the breast cancer is HER2 low, HER3 positive and ER (estrogen receptor) positive.

Another aspect of the invention is the use of an antibody which binds to human HER3 for the manufacture of a medicament for the treatment of breast cancer wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive and wherein the treatment is in combination with an antibody binding to human HER2 and inhibiting dimerization of HER2. In one preferred embodiment the breast cancer is HER2 low, HER3 positive and ER (estrogen receptor) positive.

Another aspect of the invention is the use of an antibody binding to human HER2 and inhibiting dimerization of HER2 for the manufacture of a medicament for the treatment of breast cancer wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive and wherein the treatment is in combination with an antibody which binds to human HER3. In one preferred embodiment the breast cancer is HER2 low, HER3 positive and ER (estrogen receptor) positive.

Another aspect of the invention is a method of treating a patient suffering from breast cancer wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive, the method comprising the administration of (an effective amount of) an antibody which binds to human HER3 and the co-administration of (an effective amount of) an antibody binding to human HER2 and inhibiting dimerization of HER2 . In one preferred embodiment the breast cancer is HER2 low, HER3 positive and ER (estrogen receptor) positive.

Surprisingly it was found that the combination therapy an anti-HER3 antibody described above with an antibody which binds to human HER2 and which inhibits dimerization of HER2 showed strong tumor growth inhibition of HER2 low, HER3 positive and HR (hormone receptor) positive (e.g. ER positive) breast cancers, even in tumors where the antibody which binds to human HER2 and which inhibits dimerization of HER2, only showed low to medium tumor growth inhibition when administered alone.

Description of the Figures

Figure 1A and B: Percent (%) inhibition HER3 receptor phosphorylation in MCF7 cells by anti-HER3 antibodies in different concentrations.

Figure 1C Percent (%) inhibition of HER receptor phosphorylation in Mel-Juso cells by anti-HER3 antibodies in different concentrations.

Figure 2: Western blot of total cell lysate blotted against ER, pER (Ser 118) and HER2.

Figure 3A,B and C: Western blot after immune precipitation (IP)-HER2 and blotting against ER or pER ER α from HEK 293 cells expressing HER2 and ER. 3B shows Immunoprecipitation with ant-ER and blotting against HER2 in the same cells.

Figure 4A,B and C: Western blot after immune precipitation (IP) f ER or HER3 expressing HEK 293 cells with anti HER3 blotted against ER (4A) and pER (4B). 4C shows IP with anti ER blotted gains HER3.

Figure 5: In vivo antitumor efficacy of anti-HER3-antibody Mab205.10.2 in combination with pertuzumab in a fragment based HER2 low, ER+, HER3 + breast cancer patient-derived xenograft model (HBCx-19). Fig. 5A: after 61 days (=end of treatment) Fig.5B: after 91 days (with end of treatment on day 61). Fig.5C: ex vivo analyse of MAPK at day 91 and Fig.5D: ex vivo analyse of AKT at day 91.

Figure 6: In vivo antitumor efficacy of anti-HER3-antibody Mab205.10.2 in combination with pertuzumab and the selective estrogen receptor degrader (SERD) fulvestrant in a fragment based HER2 low, ER+, HER3 + breast cancer patient-derived xenograft model (HBCx-19).

Figure 7: In vivo antitumor efficacy (reduction of lesions) of anti-HER3-antibody Mab205.10.2 in combination with pertuzumab in pretreated (second (2L) to fourth line (4L) and first line (1L) HER2 low, ER+, HER3 + metastatic breast cancer patients. All patients are HER2 low, ER+, HER3 + except patients 1071, 1072 and 1375 who are estrogen receptor negative (ER-). Results in terms of RECIST criterias (SD = Stable Disease, PR = Partial Response, CR = Complete Response, PD = Progressive Disease; ORR =Overall Response Rate, DCR Disease Control rate. The best overall RECIST responses and the line of treatment are indicated.

Figure 8: In vivo antitumor efficacy (reduction of lesions) of anti-HER3-antibody Mab205.10.2 in combination with pertuzumab in only first line (1L) HER2 low, ER+, HER3 +

- 8 -

5 metastatic breast cancer patients. All patients are HER2 low, ER+, HER3 +. Results in terms of RECIST criterias (SD = Stable Disease, PR = Partial Response, CR = Complete Response, PD = Progressive Disease; ORR =Overall Response Rate, DCR Disease Control rate. The best overall RECIST responses and the line of treatment are indicated.

Detailed Description of the Invention

10 The invention relates to an antibody which binds to human HER3 for use in the treatment of, or the manufacture of a medicament for the treatment of, or a method of treatment of, breast cancer in combination with an antibody binding to human HER2 and inhibiting dimerization of HER2, wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive. In one embodiment the breast cancer
15 is HER2 low, HER3 positive and ER (estrogen receptor) positive.

The invention also relates to an antibody binding to human HER2 and inhibiting dimerization of HER2 for use in the treatment of, or the manufacture of a medicament for the treatment of, or a method of treatment of,
20 breast cancer in combination with an antibody which binds to human HER3, wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive. In one embodiment the breast cancer is HER2 low, HER3 positive and ER (estrogen receptor) positive .

25 The invention comprises an antibody which binds to human HER3, characterized in that the heavy chain variable domain comprises a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6 or a CDR1L region of SEQ ID NO:7 for use in the combination therapies described
30 herein.

The invention further comprises an antibody which binds to human HER3 according to the invention characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9, or the light chain variable domain VL is SEQ ID NO:10, or the light chain variable

domain VL is SEQ ID NO:11; or a humanized version thereof for use in the combination therapies described herein.

5 The invention further comprises an antibody which binds to human HER3 according to the invention characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9, or the light chain variable domain VL is SEQ ID NO:10, or the light chain variable domain VL is SEQ ID NO:11 for use in the combination therapies described herein.

10 In one embodiment the antibody which binds to human HER3 according to the invention is characterized in comprising as heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6 for use in the combination therapies described herein.

15 In one embodiment the antibody which binds to human HER3 according to the invention is characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9 or the light chain variable domain VL is SEQ ID NO:11 for use in the combination therapies described herein.

20 In one embodiment the antibody which binds to human HER3 according to the invention is characterized in comprising as heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7 for use in the combination therapies described herein.

In one embodiment the antibody which binds to human HER3 according to the invention is characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:10 for use in the combination therapies described herein.

30 In one embodiment such anti-HER3 antibody is monoclonal. In one embodiment such antibody is humanized or human. In one embodiment such anti-HER3 antibody is of IgG1 or IgG4 subclass. In one embodiment such anti-HER3 antibody is a monoclonal humanized antibody of IgG1 subclass. In one embodiment such

anti-HER3 antibody is of IgG1 subclass and is characterized in that said antibody is glycosylated with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65 % or lower (in one embodiment, the amount of fucose is between 65 % and 5 %).

- 5 The invention comprises the humanized anti-HER3 antibodies Mab 205.10.1, Mab 205.10.2 and Mab 205.10.3 with their respective VH and VL or CDRs for use in the combination therapies described herein.

Antibody	VH	VL
Mab 205.10.1	SEQ ID NO: 8	SEQ ID NO: 9
Mab 205.10.2	SEQ ID NO: 8	SEQ ID NO: 10
Mab 205.10.3	SEQ ID NO: 8	SEQ ID NO: 11

Antibody	CDR3H	CDR2H	CDR1H	CDR3L	CDR2L	CDR1L
Mab 205.10.1	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 6
Mab 205.10.2	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 7
Mab 205.10.3	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 6

- 10 In one embodiment such anti-HER3 antibodies comprise constant regions of human origin e.g. SEQ ID NO:12-16, preferably of SEQ ID NO:12-13.

- The invention comprises an antibody which binds to human HER2, characterized in comprising the heavy chain variable domain VH (SEQ ID NO: 20) and the light chain variable domain VL (SEQ ID NO:21) of pertuzumab for use in the combination therapies described herein. In one embodiment the antibody which binds to human HER2 for use in the combination therapies described herein is pertuzumab.
- 15

- The term “antibody” encompasses the various forms of antibody structures including, but not being limited to, whole antibodies and antibody fragments. The antibody according to the invention is preferably a human antibody, humanized antibody, chimeric antibody, or further genetically engineered antibody as long as the characteristic properties according to the invention are retained.
- 20

“Antibody fragments” comprise a portion of a full length antibody, preferably the variable domain thereof, or at least the antigen binding site thereof. Examples of antibody fragments include diabodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. scFv antibodies are, e.g.,
5 described in Huston, J.S., *Methods in Enzymol.* 203 (1991) 46-88. In addition, antibody fragments comprise single chain polypeptides having the characteristics of a V_H domain, namely being able to assemble together with a V_L domain, or of a V_L domain binding to the respective antigen being able to assemble together with a V_H domain to a functional antigen binding site and thereby providing the properties
10 of an antibody according to the invention.

The terms “monoclonal antibody” or “monoclonal antibody composition” as used herein refer to a preparation of antibody molecules of a single amino acid composition.

The term "chimeric antibody" refers to a monoclonal antibody comprising a
15 variable region, i.e., binding region, from mouse and at least a portion of a constant region derived from a different source or species, usually prepared by recombinant DNA techniques. Chimeric antibodies comprising a mouse variable region and a human constant region are especially preferred. Such rat/human chimeric antibodies are the product of expressed immunoglobulin genes comprising DNA
20 segments encoding rat immunoglobulin variable regions and DNA segments encoding human immunoglobulin constant regions. Other forms of "chimeric antibodies" encompassed by the present invention are those in which the class or subclass has been modified or changed from that of the original antibody. Such "chimeric" antibodies are also referred to as "class-switched antibodies." Methods
25 for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques now well known in the art. See, e.g., Morrison, S.L., et al., *Proc. Natl. Acad. Sci. USA* 81 (1984) 6851-6855; US 5,202,238 and US 5,204,244.

The term "humanized antibody" or “humanized version of an antibody” refers to
30 antibodies in which the framework or "complementarity determining regions" (CDR) have been modified to comprise the CDR of an immunoglobulin of different specificity as compared to that of the parent immunoglobulin. In a preferred embodiment, the CDRs of the V_H and V_L are grafted into the framework region of human antibody to prepare the "humanized antibody." See e.g.
35 Riechmann, L., et al., *Nature* 332 (1988) 323-327; and Neuberger, M.S., et al.,

Nature 314 (1985) 268-270. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies. Human heavy and light chain variable framework regions are listed e.g. in Lefranc, M.-P., Current Protocols in Immunology (2000) - Appendix 1P A.1P.1-A.1P.37 and are accessible via IMGT, the international ImMunoGeneTics information system® (<http://imgt.cines.fr>) or via <http://vbase.mrc-cpe.cam.ac.uk>. Optionally the framework region can be modified by further mutations. Particularly preferred CDRs correspond to those representing sequences recognizing the antigens noted above for chimeric antibodies. Preferably such humanized version is chimerized with a human constant region (see e.g. Sequences SEQ ID NO:12-16). The term "humanized antibody" as used herein also comprises such antibodies which are modified in the constant region to generate the properties according to the invention, especially in regard to C1q binding and/or FcR binding, e.g. by "class switching" i.e. change or mutation of Fc parts (e.g. from IgG1 to IgG4 and/or IgG1/IgG4 mutation).

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germ line immunoglobulin sequences. Human antibodies are well-known in the state of the art (van Dijk, M.A., and van de Winkel, J.G., Curr. Opin. Chem. Biol. 5 (2001) 368-374). Human antibodies can also be produced in transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire or a selection of human antibodies in the absence of endogenous immunoglobulin production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits, A., et al., Proc. Natl. Acad. Sci. USA 90 (1993) 2551-2555; Jakobovits, A., et al., Nature 362 (1993) 255-258; Brueggemann, M.D., et al., Year Immunol. 7 (1993) 33-40). Human antibodies can also be produced in phage display libraries (Hoogenboom, H.R., and Winter, G., J. Mol. Biol. 227 (1992) 381-388; Marks, J.D., et al., J. Mol. Biol. 222 (1991) 581-597). The techniques of Cole, A., et al. and Boerner, P., et al. are also available for the preparation of human monoclonal antibodies (Cole, A., et al., Monoclonal Antibodies and Cancer Therapy, Liss, A.L., p. 77 (1985); and Boerner, P., et al., J. Immunol. 147 (1991) 86-95). As already mentioned for and humanized antibodies according to the invention the term "human antibody" as used herein also comprises such antibodies which are modified in the constant region to generate the

properties according to the invention, especially in regard to C1q binding and/or FcR binding, e.g. by “class switching” i.e. change or mutation of Fc parts (e.g. from IgG1 to IgG4 and/or IgG1/IgG4 mutation).

5 As used herein, the terms “which binds to human HER3”, “which specifically binds to human HER3”, or “anti-HER3 antibody” are interchangeable and refer to an antibody which specifically binds to the human HER3 antigen with a binding affinity of KD-value of 1.0×10^{-8} mol/l or lower at 25°C, in one embodiment of a KD-value of 1.0×10^{-9} mol/l or lower at 25°C. The binding affinity is determined with a standard binding assay at 25°C, such as surface plasmon resonance
10 technique (BIAcore®, GE-Healthcare Uppsala, Sweden). A method for determining the KD-value of the binding affinity is described in Example 2b). Thus an “antibody which binds to human HER3” as used herein refers to an antibody specifically which binds to the human HER3 antigen with a binding affinity of KD 1.0×10^{-8} mol/l or lower (in one embodiment of KD 1.0×10^{-8} mol/l - 1.0×10^{-13} mol/l) at 25°C.
15

As used herein, the terms “binding to human HER2”, “specifically binding to human HER2”, “which binds to human HER2”, “which specifically binds to human HER2”, or “anti-HER2 antibody” are interchangeable and refer to an antibody which specifically binds to the human HER2 antigen with a binding affinity of KD-value of 1.0×10^{-8} mol/l or lower at 25°C, in one embodiment of a KD-value of 1.0×10^{-9} mol/l or lower at 25°C. The binding affinity is determined with a standard binding assay at 25°C, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden). A method for determining the KD-value of the binding affinity is described in Example 2b). Thus an “antibody which binds to
20 human HER2” as used herein refers to an antibody specifically which binds to the human HER2 antigen with a binding affinity of KD 1.0×10^{-8} mol/l or lower (in one embodiment of KD 1.0×10^{-8} mol/l - 1.0×10^{-13} mol/l) at 25°C.
25

The pairing of HER receptors on the cell surface is referred to as “dimerization”. Besides its homodimerization, HER2 dimerizes with the other members of the
30 HER family, including HER1, HER3, and HER4; HER2:HER3 dimerization is believed to produce the strongest mitogenic signaling and activate 2 key pathways that regulate cell survival and growth (Mitogen-activated protein kinase (MAPK) pathway and Phosphoinositide 3-kinase (PI3K) pathway). As used herein, the term “an antibody which binding to human HER2 and inhibiting dimerization of HER2”
35 refer to an anti-HER2 antibody which specifically binds to the human HER2

antigen and which inhibits/blocks ligand-dependent HER2 homodimerization and HER2 heterodimerization with HER1, HER3, and HER4, and especially inhibits HER2/HER3 dimerization (see e.g. Perjeta® Prescribing Information. Genentech, Inc. June 2012. Baselga J, et al; N Engl J Med. 2012;366:109-119; Baselga J, et al
5 Nat Rev Cancer. 2009;9:463-475; Hynes NE, et al Nat Rev Cancer. 2005;5:341-354; Yarden Y, et al, Nat Rev Mol Cell Biol. 2001;2:127-137; Hsieh AC, et al, Br J Cancer. 2007;97:453-457; Soltoff SP, et al, Mol Cell Biol. 1994;14:3550-3558). Examples of such anti-HER2 antibodies which inhibit HER2 dimerization are described e.g. in WO 01/00245 and WO 2006/007398 wherein pertuzumab
10 (referred to as rhuMAb 2C4 or pertuzumab) is described as one example.

Human HER3 (ErbB-3, ERBB3, c-erbB-3,c-erbB3, receptor tyrosine-protein kinase erbB-3, SEQ ID NO: 17 including signal peptide) encodes a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases which also includes HER1 (also known as EGFR), HER2, and HER4 (Kraus, M.H. et al,
15 PNAS 86 (1989), 9193-9197; Plowman, G.D. et al, PNAS 87 (1990), 4905-4909; Kraus, M.H. et al, PNAS 90 (1993), 2900-2904). Like the prototypical epidermal growth factor receptor, the transmembrane receptor HER3 consists of an extracellular ligand-binding domain (ECD), a dimerization domain within the ECD, a transmembrane domain, an intracellular protein tyrosine kinase domain
20 (TKD) and a C-terminal phosphorylation domain. This membrane-bound protein has HER3 a Heregulin (HRG) binding domain within the extracellular domain but not an active kinase domain. It therefore can bind this ligand but not convey the signal into the cell through protein phosphorylation. However, it does form heterodimers with other HER family members which do have kinase activity.
25 Heterodimerization leads to the activation of the receptor-mediated signaling pathway and transphosphorylation of its intracellular domain. Dimer formation between HER family members expands the signaling potential of HER3 and is a means not only for signal diversification but also signal amplification. For example the HER2/HER3 heterodimer induces one of the most important mitogenic signals
30 via the PI3K and AKT pathway among HER family members (Sliwkowski, M.X., et al, J. Biol. Chem. 269 (1994) 14661-14665; Alimandi, M., et al, Oncogene 10 (1995) 1813-1821; Hellyer, N.J., J. Biol. Chem. 276 (2001) 42153-421561; Singer, E., J. Biol. Chem. 276 (2001) 44266-44274; Schaefer, K.L., Neoplasia 8 (2006) 613-622).

35 Anti-HER3 antibodies Mab205.10.1, Mab205.10.2, and Mab205.10.3 showed a competitive binding with the ligand Heregulin (HRG) to HER3.

Expression of HER3 gene and/or expression of its protein have been reported in numerous cancers, including prostate, bladder, and breast tumors. Alternate transcriptional splice variants encoding different isoforms have been characterized. One isoform lacks the intermembrane region and is secreted outside the cell. This form acts to modulate the activity of the membrane-bound form. Additional splice variants have also been reported, but they have not been thoroughly characterized.

The term "human HER2" according to the invention refers to 185-kDa growth factor receptor also referred to as neu and c-erbB-2 (Slamon, et al., Science 235 (1987) 177-182; Swiss-Prot P04626; SEQ ID NO:18 including signal peptide) whose function is related to neoplastic transformation in human breast cancer cells. The HER receptor will generally comprise an extracellular domain, which may bind an HER ligand; a lipophilic transmembrane domain, a conserved intracellular tyrosine kinase domain, and a carboxyl-terminal signaling domain harboring several tyrosine residues which can be phosphorylated. The extracellular domain of HER2 comprises four domains, Domain I (amino acid residues from about 1-195), Domain II (amino acid residues from about 196-320), Domain III (amino acid residues from about 321-488), and Domain IV (amino acid residues from about 489-632) (residue numbering without signal peptide). See Garrett, et al., Mol. Cell. 11 (2003) 495-505, Cho, et al., Nature 421 (2003) 756-760, Franklin, et al., Cancer Cell 5 (2004) 317-328, or Plowman, et al., Proc. Natl. Acad. Sci. 90 (1993) 1746-1750 and WO 2006/007398.

Pertuzumab (e.g. Perjeta®) is a recombinant humanized anti-HER2 monoclonal antibody used for the treatment of HER2 positive cancers. Pertuzumab binds specifically to the 2C4 epitope, a different epitope on the extracellular domain of HER2 than trastuzumab. Pertuzumab is the first in a new class of HER dimerization inhibitors (HDIs). Through its binding to the HER2 extracellular domain, pertuzumab inhibits dimerization of HER2 (especially ligand-activated heterodimerization with other HER family members), thereby inhibiting downstream signaling pathways and cellular processes associated with tumor growth and progression (Franklin, M.C., et al. Cancer Cell 5 (2004) 317-328 and Friess, T, et al. Clin Cancer Res 11 (2005) 5300-5309). Pertuzumab is a recombinant humanized version of the murine anti-HER2 antibody 2C4 (referred to as rhuMAb 2C4 or pertuzumab) and it is described together with the respective method of preparation in WO 01/00245 and WO 2006/007398.

Herein, "Pertuzumab" and "rhuMAb 2C4" refer to an antibody that binds to the 2C4 epitope and preferably comprising the variable light and variable heavy amino acid sequences disclosed in WO 2006/044908, more particularly the humanized 2C4 version 574 disclosed in Fig. 2 of WO 2006/044908. The term "Pertuzumab" encompasses all corresponding anti-HER2 antibodies that fulfill the requirements necessary for obtaining a marketing authorization as an identical or biosimilar product in a country or territory selected from the group of countries consisting of the USA, Europe and Japan. The "epitope 2C4" is the region in the extracellular domain of HER2 to which the antibody 2C4 binds. In order to screen for antibodies which bind to the 2C4 epitope, a routine cross-blocking assay such as that described in Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 2C4 epitope of HER2. Epitope 2C4 comprises residues from domain II in the extracellular domain of HER2. 2C4 and Pertuzumab bind to the extracellular domain of HER2 at the junction of domains I, II and III (Franklin et al. Cancer Cell 5:317-328 (2004)).

The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinant include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody.

The "variable domain of an antibody according to the invention" (variable domain of a light chain (V_L), variable domain of a heavy chain (V_H)) as used herein denotes each of the pair of light and heavy chain domains which are involved directly in binding the antibody to the antigen. The variable light and heavy chain domains have the same general structure and each domain comprises four framework (FR) regions whose sequences are widely conserved, connected by three "hypervariable regions" (or complementary determining regions, CDRs). The framework regions adopt a β -sheet conformation and the CDRs may form loops connecting the β -sheet structure. The CDRs in each chain are held in their three-dimensional structure by the framework regions and form together with the CDRs from the other chain the antigen binding site. The antibody's heavy and light chain CDR3 regions play a particularly important role in the binding specificity/affinity of the antibodies according to the invention and therefore provide a further object of the invention.

The term “antigen-binding portion of an antibody” when used herein refer to the amino acid residues of an antibody which are responsible for antigen-binding. The antigen-binding portion of an antibody comprises amino acid residues from the “complementary determining regions” or “CDRs”. The term “antigen-binding portion” of an antibody of the invention contains six complementarity determining regions (CDRs) which contribute in varying degrees to the affinity of the binding site for antigen. There are three heavy chain variable domain CDRs (CDRH1, CDRH2 and CDRH3) and three light chain variable domain CDRs (CDRL1, CDRL2 and CDRL3). The term “CDRH1” denotes the CDR1 region of the heavy chain variable region calculated according to Kabat. CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 mean the respective regions from the heavy (H) or light(L) chain. The extent of CDR and framework regions (FRs) is determined by comparison to a compiled database of amino acid sequences in which those regions have been defined according to variability among the sequences according to Kabat et al, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991).

The “Fc part” of an antibody is not involved directly in binding of an antibody to an antigen, but exhibit various effector functions. A “Fc part of an antibody” is a term well known to the skilled artisan and defined on the basis of papain cleavage of antibodies. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies or immunoglobulins are divided in the classes: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG1, IgG2, IgG3, and IgG4, IgA1, and IgA2. According to the heavy chain constant regions the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The Fc part of an antibody is directly involved in ADCC (antibody-dependent cell-mediated cytotoxicity) and CDC (complement-dependent cytotoxicity) based on complement activation, C1q binding and Fc receptor binding. The term “complement-dependent cytotoxicity (CDC)” denotes a process initiated by binding of complement factor C1q to the Fc part of most IgG antibody subclasses. Binding of C1q to an antibody is caused by defined protein-protein interactions at the so called binding site. Such binding sites are known in the state of the art and described e.g. by Boackle, R.J., et al., Nature 282 (1979) 742-743, Lukas, T.J., et al., J. Immunol. 127 (1981) 2555-2560, Brunhouse, R., and Cebra, J.J., Mol. Immunol. 16 (1979) 907-917, Burton, D.R., et al., Nature 288 (1980) 338-344, Thommesen, J.E., et al., Mol. Immunol. 37 (2000) 995-1004, Idusogie, E.E., et al., J. Immunol. 164 (2000) 4178-4184, Hezareh, M., et al., J.

Virology 75 (2001) 12161-12168, Morgan, A., et al., Immunology 86 (1995) 319-324, EP 0 307 434. Such binding sites are e.g. L234, L235, D270, N297, E318, K320, K322, P331 and P329 (numbering according to EU index of Kabat, E.A., see below). Antibodies of subclass IgG1, IgG2 and IgG3 usually show complement activation and C1q and C3 binding, whereas IgG4 do not activate the complement system and do not bind C1q and C3.

In one embodiment the antibody according to the invention comprises a Fc part derived from human origin and preferably all other parts of the human constant regions. As used herein the term "Fc part derived from human origin" denotes a Fc part which is either a Fc part of a human antibody of the subclass IgG1, IgG2, IgG3 or IgG4, e.g. a Fc part from human IgG1 subclass, a mutated Fc part from human IgG1 subclass (preferably with a mutation on L234A + L235A), a Fc part from human IgG4 subclass or a mutated Fc part from human IgG4 subclass (preferably with a mutation on S228P). Preferred are the human heavy chain constant regions from human IgG1 subclass.

In one embodiment the anti-HER3 according to the invention is of human IgG1 subclass. In one embodiment the anti-HER2 according to the invention is of human IgG1 subclass. In one embodiment the anti-HER3 and the anti-HER2 antibody according to the invention are both of human IgG1 subclass.

In one embodiment the anti-HER3 and the anti-HER2 antibody according to the invention are both characterized in that the constant chains are of human origin. Such constant chains are well known in the state of the art and e.g. described by Kabat, E.A., (see e.g. Johnson, G. and Wu, T.T., Nucleic Acids Res. 28 (2000) 214-218). For example, a useful human heavy chain constant region comprises an amino acid sequence of SEQ ID NO: 13. For example, a useful human light chain constant region comprises an amino acid sequence of a kappa-light chain constant region of SEQ ID NO: 12.

The term "amino acid" as used within this application denotes the group of naturally occurring carboxy α -amino acids comprising alanine (three letter code: ala, one letter code: A), arginine (arg, R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V).

The terms “nucleic acid” or “nucleic acid molecule”, as used herein, are intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA. A nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid. For example, DNA for a presequence or secretory leader is operable linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operable linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operable linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operable linked” means that the DNA sequences being linked are colinear, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. As used herein, the expressions “cell”, “cell line”, and “cell culture” are used interchangeably and all such designations include progeny. Thus, the words “transformants” and “transformed cells” include the primary subject cell and cultures derived there from without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

The anti-HER3 antibody described herein is preferably characterized in that the constant chains are of human origin. Such constant chains are well known in the state of the art and described, e.g., by Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991). For example, a useful human light chain constant region comprises an amino acid sequence of a kappa-light chain constant region of SEQ ID NO:12. For example, useful human heavy chain constant region comprises SEQ ID NO:13 to 16.

In another aspect, an anti-HER3 antibody for the respective combination therapy is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:8 and SEQ ID NO:10, respectively,; and having one or more of the following properties (determined in assays as described in Example 3 and 2):

- 5 - the anti-HER3 antibody inhibits the HER3 phosphorylation in tumor cells such as MCF7 cells, FaDu cells or Mel-Juso cell (in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in MCF7 cells of at least 80% (in one embodiment at least 90%) at a concentration of 1.0 µg/ml ; in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in FaDu cells of at least 80% (in one embodiment at least 90%) at a concentration of 0.1 µg/ml ; in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in Mel-Juso cells of at least 60% (in one embodiment at least 70%) at a concentration of 0.1 µg/ml)
- 10 - the anti-HER3 antibody inhibits the AKT phosphorylation in tumor cells such as Mel-Juso cell (in one embodiment the anti-HER3 antibody inhibits the AKT phosphorylation in Mel-Juso cells with an IC₅₀ value of less than 0.50 µg/ml, in one embodiment with IC₅₀ value of less than 0.35 µg/ml)
- 15 - the anti-HER3 antibody inhibits the proliferation of tumor cells such as MDA-MB-175 cells (in one embodiment the anti-HER3 antibody inhibits the proliferation of MDA-MB-175 cells with an IC₅₀ value of less than 10 µg/ml)
- 20 - the anti-HER3 antibody binds to HER3 with a K_D value of less than 5.0x 10⁻⁹ M, in one embodiment with a K_D value of less than 3.0x 10⁻⁹ M.

In another aspect, an anti-HER3 antibody for the respective combination therapy is a bispecific anti-HER3/anti-HER1 antibody as described in US 2010/0255010. In one embodiment, the bispecific anti-HER3/anti-HER1 antibody is characterized comprising by the characteristic amino acid sequences disclosed in

25 US 2010/0255010, i.e. A) (a) HVR-H1 comprising the amino acid sequence of LSGDWIH; (b) HVR-H2 comprising the amino acid sequence of VGEISAAGGYTD; and (c) HVR-H3 comprising the amino acid sequence of ARESRVSFEEAMDY; and (d) HVR-L1 comprising the amino acid sequence of NIATDVA; (e) HVR-L2 comprising the amino acid sequence of SASF; and (f)

30 HVR-L3 comprising the amino acid sequence of SEPEPYT, or B) (a) a heavy chain variable domain with the amino acid sequence of SEQ ID NO: 30 as disclosed in US2010/0255010; (b) a light chain variable domain with the amino acid sequence of SEQ ID NO: 29 as disclosed in US2010/0255010.

The term “antibody-dependent cellular cytotoxicity (ADCC)” refers to lysis of human target cells by an antibody according to the invention in the presence of effector cells. ADCC is measured preferably by the treatment of a preparation of HER3 expressing cells with an antibody according to the invention in the presence of effector cells such as freshly isolated PBMC or purified effector cells from buffy coats, like monocytes or natural killer (NK) cells or a permanently growing NK cell line.

Cell-mediated effector functions like ADCC of monoclonal antibodies can be enhanced by engineering their oligosaccharide component as described in Umana, P., et al., *Nature Biotechnol.* 17 (1999) 176-180, and US 6,602,684. IgG1 type antibodies, the most commonly used therapeutic antibodies, are glycoproteins that have a conserved N-linked glycosylation site at Asn297 in each CH2 domain. The two complex biantennary oligosaccharides attached to Asn297 are buried between the CH2 domains, forming extensive contacts with the polypeptide backbone, and their presence is essential for the antibody to mediate effector functions such as antibody dependent cellular cytotoxicity (ADCC) (Lifely, M.R., et al., *Glycobiology* 5 (1995) 813-822; Jefferis, R., et al., *Immunol. Rev.* 163 (1998) 59-76; Wright, A., and Morrison, S.L., *Trends Biotechnol.* 15 (1997) 26-32). Umana, P., et al., *Nature Biotechnol.* 17 (1999) 176-180 and WO 99/54342 showed that overexpression in Chinese hamster ovary (CHO) cells of $\beta(1,4)$ -N-acetylglucosaminyltransferase III (“GnTIII”), a glycosyltransferase catalyzing the formation of bisected oligosaccharides, significantly increases the in vitro ADCC activity of antibodies. Alterations in the composition of the Asn297 carbohydrate or its elimination affect also binding to Fc γ R and C1q (Umana, P., et al., *Nature Biotechnol.* 17 (1999) 176-180; Davies, J., et al., *Biotechnol. Bioeng.* 74 (2001) 288-294; Mimura, Y., et al., *J. Biol. Chem.* 276 (2001) 45539-45547; Radaev, S., et al., *J. Biol. Chem.* 276 (2001) 16478-16483; Shields, R.L., et al., *J. Biol. Chem.* 276 (2001) 6591-6604; Shields, R.L., et al., *J. Biol. Chem.* 277 (2002) 26733-26740; Simmons, L.C., et al., *J. Immunol. Methods* 263 (2002) 133-147).

Methods to enhance cell-mediated effector functions of monoclonal antibodies via glycoengineering are reported e.g. in WO 2005/044859, WO 2004/065540, WO2007/031875, Umana, P., et al., *Nature Biotechnol.* 17 (1999) 176-180, WO 99/154342, WO 2005/018572, WO 2006/116260, WO 2006/114700, WO 2004/065540, WO 2005/011735, WO 2005/027966, WO 1997/028267, US 2006/0134709, US 2005/0054048, US 2005/0152894, WO 2003/035835 and WO 2000/061739 or e.g. in Niwa, R., et al., *J. Immunol. Methods* 306 (2005) 151-

160; Shinkawa, T., et al, J Biol Chem, 278 (2003) 3466–3473; WO 03/055993 and US 2005/0249722.

5 In one embodiment of the invention, the antibody according to the invention is afucosylated which means the antibody is glycosylated (if it comprises an Fc part of IgG1 subclass) with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 80% or lower (Numbering according to Kabat), e.g. between 80% and 1 %. In another embodiment is the amount of fucose within said sugar chain is 65% or lower, in one embodiment between 5% and 65%, in one embodiment from 0% to 65%, and in one embodiment the amount of fucose within
10 said sugar chain is 0%. Such antibodies are referred to in the following as “afucosylated antibodies” or “non-fucosylated antibodies”. Such afucosylated antibodies show enhanced ADCC whereas other antibody properties remain substantially unaffected.

15 In a further embodiment the amount of N-glycolylneuraminic acid (NGNA) is 1% or less and/or the amount of N-terminal alpha-1,3-galactose is 1% or less within said sugar chain. The sugar chain show preferably the characteristics of N-linked glycans attached to Asn297 of an antibody recombinantly expressed in a CHO cell.

20 “Asn297” according to the invention means amino acid asparagine located at about position 297 in the Fc region. Based on minor sequence variations of antibodies, Asn297 can also be located some amino acids (usually not more than ± 3 amino acids) upstream or downstream of position 297, i.e. between position 294 and 300.

25 The term “the sugar chains show characteristics of N-linked glycans attached to Asn297 of an antibody recombinantly expressed in a CHO cell” denotes that the sugar chain at Asn297 of the full length parent antibody according to the invention has the same structure and sugar residue sequence except for the fucose residue as those of the same antibody expressed in unmodified CHO cells, e.g. as those reported in WO 2006/103100.

The term “NGNA” as used within this application denotes the sugar residue N-glycolyl-neuraminic acid.

30 Glycosylation of human IgG1 occurs at Asn297 as core fucosylated biantennary complex oligosaccharide glycosylation terminated with up to two Gal residues. Human constant heavy chain regions of the IgG1 subclass are reported in detail by Kabat, E., A., et al., Sequences of Proteins of Immunological Interest, 5th Ed.

Public Health Service, National Institutes of Health, Bethesda, MD. (1991), and by Brueggemann, M., et al., J. Exp. Med. 166 (1987) 1351-1361; Love, T.W., et al., Methods Enzymol. 178 (1989) 515-527. These structures are designated as G0, G1 (α -1,6- or α -1,3-), or G2 glycan residues, depending from the amount of terminal Gal residues (Raju, T.S., Bioprocess Int. 1 (2003) 44-53). CHO type glycosylation of antibody Fc parts is e.g. described by Routier, F.H., Glycoconjugate J. 14 (1997) 201-207. Antibodies which are recombinantly expressed in non-glycomodified CHO host cells usually are fucosylated at Asn297 in an amount of at least 85%. The modified oligosaccharides of the full length parent antibody may be hybrid or complex. Preferably the bisected, reduced/not-fucosylated oligosaccharides are hybrid. In another embodiment, the bisected, reduced/not-fucosylated oligosaccharides are complex.

According to the invention "amount of fucose" means the amount of said sugar within the sugar chain at Asn297, related to the sum of all glycostructures attached to Asn297 (e.g. complex, hybrid and high mannose structures) measured by MALDI-TOF mass spectrometry (e.g. in LC/MS system) and calculated as average value (see e.g. WO 2008/077546). The relative amount of fucose is the percentage of fucose-containing structures related to all glycostructures identified in an N-Glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures, resp.) by MALDI-TOF.

The antibodies according to the invention are preferably produced by recombinant means. Such methods are widely known in the state of the art and comprise protein expression in prokaryotic and eukaryotic cells with subsequent isolation of the antibody polypeptide and usually purification to a pharmaceutically acceptable purity. For the protein expression nucleic acids encoding light and heavy chains or fragments thereof are inserted into expression vectors by standard methods. Expression is performed in appropriate prokaryotic or eukaryotic host cells, such as CHO cells, NS0 cells, SP2/0 cells, HEK293 cells, COS cells, yeast, or E. coli cells, and the antibody is recovered from the cells (from the supernatant or after cells lysis). Recombinant production of antibodies is well-known in the state of the art and described, for example, in the review articles of Makrides, S.C., Protein Expr. Purif. 17 (1999) 183-202; Geisse, S., et al., Protein Expr. Purif. 8 (1996) 271-282; Kaufman, R.J., Mol. Biotechnol. 16 (2000) 151-161; Werner, R.G., Drug Res. 48 (1998) 870-880. The antibodies may be present in whole cells, in a cell lysate, or in a partially purified, or substantially pure form. Purification is performed in order to eliminate other cellular components or other contaminants, e.g., other cellular

nucleic acids or proteins, by standard techniques, including, column chromatography and others well known in the art (see Ausubel, F., et al., ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987)). Expression in NS0 cells is described by, e.g.,
5 Barnes, L.M., et al., Cytotechnology 32 (2000) 109-123; Barnes, L.M., et al., Biotech. Bioeng. 73 (2001) 261-270. Transient expression is described by, e.g., Durocher, Y., et al., Nucl. Acids. Res. 30 (2002) E9. Cloning of variable domains is described by Orlandi, R., et al., Proc. Natl. Acad. Sci. USA 86 (1989) 3833-3837; Carter, P., et al., Proc. Natl. Acad. Sci. USA 89 (1992) 4285-4289;
10 Norderhaug, L., et al., J. Immunol. Methods 204 (1997) 77-87. A preferred transient expression system (HEK 293) is described by Schlaeger, E.-J. and Christensen, K., in Cytotechnology 30 (1999) 71-83, and by Schlaeger, E.-J., in J. Immunol. Methods 194 (1996) 191-199. Monoclonal antibodies are suitably separated from the culture medium by conventional immunoglobulin purification
15 procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. DNA and RNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures. The hybridoma cells can serve as a source of such DNA and RNA. Once isolated, the DNA may be inserted into expression vectors,
20 which are then transfected into host cells, such as HEK 293 cells, CHO cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of recombinant monoclonal antibodies in the host cells. Antibodies obtainable from said cell lines are preferred embodiments of the invention. Afucosylated antibodies are preferably prepared via glycoengineering as described
25 above.

The heavy and light chain variable domains according to the invention are combined with sequences of promoter, translation initiation, constant region, 3' untranslated region, polyadenylation, and transcription termination to form expression vector constructs. The heavy and light chain expression constructs can
30 be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a single host cell expressing both chains.

In one aspect of the invention the antibodies of the combination are administered as a pharmaceutical composition comprising the respective antibody. In another
35 aspect, the present invention provides a composition, e.g. a pharmaceutical

composition, containing an antibody according to the present invention, formulated together with a pharmaceutical carrier.

As used herein, “pharmaceutical carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption
5 delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g. by injection or infusion).

A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route
10 and/or mode of administration will vary depending upon the desired results. To administer a compound of the invention by certain routes of administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a
15 diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Pharmaceutical carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art.

The phrases “parenteral administration” and “administered parenterally” as used
20 herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intra-articular,
25 subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

The term “breast cancer” as used herein including any metastatic or refractory versions.

Another aspect of the invention is an anti-HER3-antibody according to the
30 invention for the treatment of cancer in combination with an antibody which binds to human HER2 and which inhibits dimerization of HER2, wherein the cancer is a HER2 low, HER3 positive and HR (hormone receptor) positive (e.g. ER positive) breast cancer.

Another aspect of the invention is the use of an antibody which binds to human HER3 for the manufacture of a medicament for the treatment of breast cancer in combination with an antibody which binds to human HER2 and which inhibits dimerization of HER2, wherein the cancer is a HER2 low, HER3 positive and HR (hormone receptor) positive (e.g. ER positive) cancer.

Another aspect of the invention is a method of treatment of a patient suffering from breast cancer by administering an anti-HER3-antibody according to the invention to said patient in the need of such treatment in combination with an antibody which binds to human HER2 and which inhibits dimerization of HER2, wherein the cancer is a HER2 low, HER3 positive and HR (hormone receptor) positive (e.g. ER positive) cancer.

In one embodiment, a) the anti-HER3 antibody used in this combination is characterized in comprising as VH an amino acid sequence of SEQ ID NO:8 and as VL an amino acid sequence of SEQ ID NO: 10, b) the anti-HER2 antibody used in this combination is pertuzumab, and c) the cancer is HER2 low, HER3 positive and HR (hormone receptor) positive (e.g. ER positive) breast cancer.

In the context of the combination therapy of an anti-HER3 antibody with an anti-HER2 antibody, wherein the anti-HER2 antibodies inhibits HER2 dimerization, the term "HER3 positive", "HER3+" or "HER3 expression" refers to a breast cancer or tumorous tissue which comprises cells which are characterized by HER3 protein expression and/or gene amplification. This means that the respective breast cancer samples show a detectable level of the HER3 protein expression and/or gene amplification. The expression level of HER3 may be detected by an immunohistochemical method, whereas said HER3 gene amplification status can be measured with in situ hybridization methods, like fluorescence in situ hybridization techniques (FISH). Corresponding assays and kits are well known in the art, for protein expression assays as well as for the detection of gene amplifications. The expression level of HER3 can, inter alia, be detected by an immunohistochemical method. Such methods are well known in the art (see e.g. analogous methods and test for HER2 expression levels below or e.g. WO 2015/049355). Other methods like qRT-PCR might be used to detect levels of HER3 gene amplification. In one preferred embodiment the term "HER3 positive", "HER3+" or "HER3 expression" refers to the HER3 protein expression detected by an immunohistochemical method, which means the respective breast cancer

samples show a detectable level of the HER3 protein in an corresponding immunohistochemistry (IHC) assay.

In the context of the combination therapy of an anti-HER3 antibody with an anti-HER2 antibody, wherein the anti-HER2 antibodies inhibits HER2 dimerization, the term “HER2 low” cancer also referred to as “HER2 low cancer” as used herein refers to a cancer or tumorous tissue which comprises cells which express low levels of HER2. Thus, HER2 low cancer cells do not overexpress HER2 (as defined for HER2-positive cancer) and are not negative for HER2 expression. For the purpose of the present invention, “HER2 low cancer” is a cancer with an immunohistochemistry (IHC) score of 2+ and an *in situ* hybridization (ISH) amplification ratio <2.0 (i.e. is ISH-negative) or an immunohistochemistry (IHC) score of 1+ and an *in situ* hybridization (ISH) amplification ratio <2.0 (i.e. is ISH-negative). Accordingly, HER2 low cancer is present if a low (IHC 1+) or moderate (IHC 2+) HER2 (protein) expression level detected e.g. by immunohistochemical methods and no HER2 gene amplification, detected by in-situ-hybridization (ISH negative, like a HER2 gene copy ≤ 4 copies of the HER2 gene per tumor cell or ratio of < 2.0 for the number of HER2 gene copies to the number of signals for CEP17.), is found in samples obtained from the patients such as breast tissue biopsies or breast tissue resections or in tissue derived from metastatic sites. In one embodiment “HER2 low cancer” is defined as an immunohistochemistry (IHC) score of HER2(2+) and ISH negative or immunohistochemistry (IHC) score of HER2(1+) and ISH negative (IHC 1+/ISH-negative or IHC 2+/ISH-negative).

The expression level of HER2 may be detected by an immunohistochemical method, whereas said HER2 gene amplification status can be measured with in situ hybridization methods, like fluorescence in situ hybridization techniques (FISH). Corresponding assays and kits are well known in the art, for protein expression assays as well as for the detection of gene amplifications. Alternatively other methods like qRT-PCR may be used to detect levels of HER2 gene expression.

The expression level of HER2 can, inter alia, be detected by an immunohistochemical method. Such methods are well known in the art and corresponding commercial kits are available. Exemplary kits which may be used in accordance with the present invention are, inter alia, HerceptTest™ produced and distributed by the company Dako or the test called Ventana Pathway™. The level of HER2 protein expression may be assessed by using the reagents provided with and following the protocol of the HerceptTest™. A skilled person will be aware of

further means and methods for determining the expression level of HER2 by immunohistochemical methods; see for example WO 2005/117553. Therefore, the expression level of HER2 can be easily and reproducibly determined by a person skilled in the art without undue burden. However, to ensure accurate and reproducible results, the testing must be performed in a specialized laboratory, which can ensure validation of the testing procedures.

The expression level of HER2 can be classified in a low expression level, an intermediate expression level and a high expression level. In context of this invention it is preferred that HER2 low disease is defined by a low or weak expression level of HER2 (e.g. HER2(1+ or 2+) by IHC) and a negative ISH result, for example determined in a sample of a cancer patient. Therefore parallel testing using immunohistochemistry and in situ hybridisation is preferred.

The recommended scoring system to evaluate the IHC staining patterns in breast cancer which reflect the expression levels of HER2 designated herein HER2(0), HER2(+), HER2(++) and HER2(+++), is as follows:

The below IHC staining patterns are recommended for determining HER2 status in breast cancer (see Dako HercepTest™ package insert).

Staining Intensity Score	Staining Pattern	HER2 overexpression assessment
0	No staining is observed or membrane staining is observed in < 10 % of the tumor cells	negative
1+	A faint/barely perceptible membrane staining is detected in > 10 % of the tumor cells. The cells are only stained in part of their membrane.	negative
2+	A weak to moderate complete membrane staining is detected in > 10 % of the tumor cells.	weakly positive.
3+	A strong complete membrane staining is detected in > 10 % of the tumor cells.	strongly positive

The above IHC staining patterns are routinely used in determining HER2 status in breast cancer. The terms HER2(+), HER2(++) and HER2(+++) used herein are equivalent to the terms HER2(1+), HER2(2+) and HER2(3+). A “low HER2 protein expression level” used in context of this invention corresponds to a 1+ score (“negative assessment” according to the table shown herein above), and a 2+ score “weakly positive”. As described herein above in detail, the evaluation of the

protein expression level (i.e. the scoring system as shown in the table) is based on results obtained by immunohistochemical methods. As a standard or routinely, the HER2 status is, accordingly, performed by immunohistochemistry with one of two FDA-approved commercial kits available; namely the Dako Herceptest™ and the Ventana Pathway™. These are semi-quantitative assays which stratify expression levels into 0 (<20,000 receptors per cell, no expression visible by IHC staining), 1+ (~100,000 receptors per cell, partial membrane staining, < 10% of cells overexpressing HER2), 2+ (~500,000 receptors per cell, light to moderate complete membrane staining, > 10% of cells overexpressing HER2), and 3+ (~2,000,000 receptors per cell, strong complete membrane staining, > 10% of cells overexpressing HER2).

Alternatively, further methods for the evaluation of the protein expression level of HER2 may be used, e.g. Western Blots, ELISA-based detection systems and so on.

The below IHC staining patterns are recommended for determining HER2 status in gastric cancer (see Dako Herceptest package insert):

Staining Intensity Score	Surgical specimen - staining pattern	Biopsy specimen – staining pattern	HER2 Overexpression Assessment
0	No reactivity or no membranous reactivity in < 10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
1+	Faint / barely perceptible membranous reactivity in $\geq$ 10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster ($\geq$ 5 cells) with a faint / barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in $\geq$ 10% of tumour cells	Tumour cell cluster ($\geq$ 5 cells) with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal

Staining Intensity Score	Surgical specimen - staining pattern	Biopsy specimen – staining pattern	HER2 Overexpression Assessment
3+	Strong complete, basolateral or lateral membranous reactivity in $\geq 10\%$ of tumour cells	Tumour cell cluster (≥ 5 cells) with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

HER2 low disease is defined by a low or weak expression level of HER2 (e.g. HER2(1+ or 2+) by IHC) and a negative ISH result.

In contrast HER2 positive disease is defined by a high expression level of HER2 as HER2 (3+) by IHC or HER2 (2+) by IHC and positive ISH result. And HER2 negative disease is defined as HER2 (0) by IHC and a negative ISH result.

In accordance with the above, the sample to be assessed can be (obtained) from a patient with HER2 low cancer as defined above. For example, the sample may be obtained from a tumorous tissue, (a) tumor(s) and, accordingly, is (a) tumor cell(s) or (a) tumor tissue(s) suspected of being HER2 expressing tumour, like a breast tumor. A person skilled in the art is in the position to identify such tumors and/or individuals/patients suffering from corresponding cancer using standard techniques known in the art and methods disclosed herein. Generally, said tumor cell or cancer cell may be obtained from any biological source/organism, particularly any biological source/organism, suffering from the above-mentioned cancer. In context of this invention particular useful cells are, preferably, human cells. These cells can be obtained from e.g. biopsies or from biological samples. The tumor/cancer/tumor cell/cancer cell is a solid tumor/ cancer/tumor cell/cancer cell. In accordance with the above, the cancer/tumor cell may be a breast cancer/tumor cell or said sample comprises a cancer/tumor cell, such as a breast cancer/tumor cell. In line with the above, said tumor/cancer may be a breast tumor/cancer.

In the context of the combination therapy of an anti-HER3 antibody with anti-HER2 antibody, wherein the anti-HER2 antibody inhibits HER2 dimerization, the term “Hormone receptor (HR) positive or HR+” as used herein refers to Estrogen Receptor (ER) positive and/or Progesterone Receptor (PgR)-positive cancer/tumours tissue, such as Breast Cancer. Breast Cancer are considered

positive for ER or PgR if finding of $\geq 1\%$ of tumor cell nuclei are immunoreactive, as determined by Immunohistochemistry (IHC). Breast Cancer are considered negative for ER or PgR if it is detected that $< 1\%$ of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER or PgR (positive intrinsic controls can be detected), as determined by IHC. Breast Cancer are considered uninterpretable for ER or PgR if it is detected that no tumor nuclei are immunoreactive and that internal epithelial elements present in the sample or separately submitted from the same sample lack any nuclear staining, as determined by IHC.

10 In one preferred embodiment in the combination therapy of an HER3 antibody with an anti-HER2 antibody, wherein the anti-HER2 antibody inhibits HER2 dimerization, the breast cancer is estrogen receptor (ER) positive (ER+).

The term "Estrogen receptor (ER) positive or ER+" according to the invention refers to breast cancer which is considered positive for ER if finding of $\geq 1\%$ of tumor cell nuclei are immunoreactive, as determined by Immunohistochemistry (IHC). Breast cancer is considered negative for ER if finding of $< 1\%$ of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER (positive intrinsic controls are seen), as determined by IHC. Breast cancer is considered uninterpretable for ER if finding that no tumor nuclei are immunoreactive and that internal epithelial elements present in the sample or separately submitted from the same sample lack any nuclear staining, as determined by IHC.

25 Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

30 Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration,

the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

The composition must be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier preferably is an isotonic buffered saline solution.

Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition.

The term "treating" as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, or preventing, either partially or completely, the growth of tumors, tumor metastases, or other cancer-causing or neoplastic cells in a patient. The term "treatment" as used herein, unless otherwise indicated, refers to the act of treating.

The phrase "a method of treating" or its equivalent, when applied to, for example, cancer refers to a procedure or course of action that is designed to reduce or eliminate the number of cancer cells in a patient, or to alleviate the symptoms of a cancer. "A method of treating" cancer or another proliferative disorder does not necessarily mean that the cancer cells or other disorder will, in fact, be eliminated, that the number of cells or disorder will, in fact, be reduced, or that the symptoms of a cancer or other disorder will, in fact, be alleviated. Often, a method of treating cancer will be performed even with a low likelihood of success, but which, given the medical history and estimated survival expectancy of a patient, is nevertheless deemed an overall beneficial course of action.

It is self-evident that the antibodies are administered to the patient in therapeutically effective amount which is the amount of the subject compound or combination that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

The term “in combination with” refers to the “co-administration” or “co-administering” of the anti-HER3 antibody which is administered additionally to the anti-HER2 antibody in either order. The “co-administration” means that the first antibody is administered additionally to the second antibody either simultaneously or sequentially. The co-administration can be simultaneous or sequential in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. When both antibodies are administered simultaneously the dose is administered on the same day in one administration, e.g. during one continuous infusion. When both antibodies are administered sequentially the dose is administered either on the same day in two separate administrations, e.g. two separate continuous infusions, or one of the antibodies is administered on day 1 and the second antibody is administered on day 2 to day 7, preferably on day 2 to 4. The terms “co-administration” or “co-administering” with respect to the maintenance doses of the first antibody and the second antibody mean that the maintenance doses can be either administered simultaneously, e.g. during one continuous infusion, if the treatment cycle is appropriate for both antibodies. Or the maintenance doses are administered sequentially, either within one or several days, e.g. the maintenance dose of the first antibody is administered every 3 weeks, and the maintenance dose of the second is administered every 2 weeks. Also other treatment cycles /usually from 1 to 4 weeks, preferably from 2 to 3 weeks, may be used for both antibodies.

The amount of antibody co-administration and the timing of administration may depend on the type (species, gender, age, weight, etc.) and condition of the patient being treated and the severity of the disease or condition being treated. Usually typical dosages antibodies are used.

For example, the dosages for administration of the anti-HER3 antibody according to the invention can be about 400 to 600 mg of antibody by one or more separate administrations, or by continuous infusion, typically in a 2 to 4 weeks cycle, and especially in a 3 weeks cycle. In a preferred aspect, the anti-HER3 antibody is administered every two to three weeks, at a dose ranged from about 400 to 600 mg, in one preferred embodiment at a dose of 500 mg every 3 weeks administered as continuous infusion until disease progression is detected. It was detected that by reducing the dose of the anti-HER3 antibody according to the invention from 1000 mg every 3 weeks to 500 mg every 3 weeks the side effect of severe diarrhea could be significantly reduced, while maintaining the effectiveness of the antibody

combination with the anti-HER2 antibody according to the invention (which was dosed at 420 mg every 3 weeks)

For example, the dosages for administration of the anti-HER2 antibody according to the invention can be about 300 to 500 mg of antibody by one or more separate administrations, or by continuous infusion, typically in a 2 to 4 weeks cycle, and especially in a 3 weeks cycle. In a preferred aspect, the anti-HER2 antibody is administered every two to three weeks, at a dose ranged from about 300 to 500 mg, in on preferred embodiment at a dose of 420 mg every 3 weeks administered as continuous infusion until disease progression is detected.

In the context of this invention, additional chemotherapeutic agents may be used in combination treatment of the present invention. Such agents include, for example:

- a) a taxane or taxane derivative like paclitaxel (e.g. Taxol®) and paclitaxel derivatives (like nab-paclitaxel (protein-bound paclitaxel) (e.g. Abraxane®) or docosahexaenoic acid (DHA) -paclitaxel, (e.g. Taxoprexin®)), or docetaxel (e.g. Taxotere®),
- b) cyclophosphamide (CTX) (e.g. Cytosan®),
- c) cisplatin (e.g. Platinol®) or carboplatin (e.g. Paraplatin® and Paraplatin-AQ®),
- d) mitomycin C (e.g. Mutamycin®),
- e) methotrexate (MTX) (e.g. Trexall®),
- f) 5-fluorouracil (5-FU) (e.g. Adrucil®) or capecitabine (e.g. Xeloda®),
- g) doxorubicin (DXR) (e.g. Adriamycin®) or its liposomal formulation (doxorubicin lipo) (e.g. Doxil®), or daunorubicin (e.g. Cerubidine®) or its liposomal formulation (daunorubicin lipo) (e.g. Daunoxome®),
- h) gemcitabine (e.g. Gemzar®),
- i) mitoxantrone (e.g. Novantrone ®),
- j) vinorelbine (e.g. Navelbine®),

k) eribulin (e.g. Halaven®), and

l) ixabepilone (e.g. Ixemptra®).

In the context of this invention, additional an anti-hormonal agents may be used in combination treatment of the present invention. Such agents include, for example:

5 a) a selective estrogen receptor degrader (SERD) like e.g. fulvestrant, or b) a selective estrogen receptor modulators (SERM), such as tamoxifen, raloxifene, toremifene or lasofoxifene, or c) an aromatase inhibitor like e.g. letrozole, anastrozole or exemestane).

The use of the chemotherapeutic or anti-hormonal agents described above in

10 chemotherapeutic/ anti-hormonal regimens is generally well characterized in the cancer therapy arts, and their use herein falls under the same considerations for monitoring tolerance and effectiveness and for controlling administration routes and dosages, with some adjustments. For example in breast cancer disease with metastases in organs like the liver or lung, a high tumor burden and/or relapse after

15 anti-hormonal therapy (typically anti-estrogen treatment), or rapidly progressing disease, a chemotherapeutic agent might be co-administered with the HER3/HER2 antibody combination described herein. In breast cancer disease with only metastases in bone (and not in organs) and/or lower tumor burden anti-hormonal therapy (typically an anti-estrogen treatment), or slowly progressing disease, an

20 anti-hormonal agent might be co-administered with the HER3/HER2 antibody combination described herein. The actual dosages of the agents may vary depending upon the patient's cultured cell response determined by using histoculture methods. Generally, the dosage will be reduced compared to the amount used in the absence of additional other agents. The “co-administration” or

25 “co-administering” means that the chemotherapeutic or anti-hormonal agent is administered additionally to the antibody combination either simultaneously or sequentially. The co-administration can be simultaneous or sequential in either order, wherein preferably there is a time period while all active agents simultaneously exert their biological activities.

30 The following is a list of commonly used chemotherapy for breast cancer:

CMF: cyclophosphamide, methotrexate, and 5-fluorouracil given 4-weekly for 6 cycles

FAC (or CAF): 5-fluorouracil, doxorubicin, cyclophosphamide given 3-weekly for 6 cycles

AC (or CA): Adriamycin (doxorubicin) and cyclophosphamide given 3-weekly for 4 cycles

- 5 AC-Taxol: AC given 3-weekly for 4 cycles followed by paclitaxel given either 3-weekly for 4 cycles or weekly (at a smaller dose) for 12 weeks

TAC: Taxotere (docetaxel), Adriamycin (doxorubicin), and cyclophosphamide given 3-weekly for 4-6 cycles

FEC: 5-fluorouracil, epirubicin and cyclophosphamide given 3-weekly for 6 cycles

- 10 FECD: FEC given 3-weekly for 3 cycles followed by docetaxel given 3-weekly for 3 cycles

TC: Taxotere (docetaxel) and cyclophosphamide given 3-weekly for 4 or 6 cycles

The following is a list of commonly used anti-hormonal therapy for breast cancer:

- 15 Fulvestrant, exemestane alone or in combination with everolimus, anastrozole, letrozole, tamoxifene. Recently the palbociclib has been approved in combination with letrozole and fulvestrant.

- 20 Typical dosages of an effective agent can be in the ranges recommended by the manufacturer, and where indicated by in vitro responses or responses in animal models, can be reduced by up to about one order of magnitude concentration or amount. Thus, the actual dosage will depend upon the judgment of the physician, the condition of the patient, and the effectiveness of the therapeutic method based on the in vitro responsiveness of the primary cultured malignant cells or histocultured tissue sample, or the responses observed in the appropriate animal models.

- 25 In the context of this invention, an effective amount of ionizing radiation may be carried out and/or a radiopharmaceutical may be used in addition to combination treatment of the present invention. The source of radiation can be either external or internal to the patient being treated. When the source is external to the patient, the therapy is known as external beam radiation therapy (EBRT). When the source of radiation is internal to the patient, the treatment is called brachytherapy (BT).
- 30

Radioactive atoms for use in the context of this invention can be selected from the group including, but not limited to, radium, cesium-137, iridium-192, americium-241, gold-198, cobalt-57, copper-67, technetium-99, iodine-123, iodine-131, and indium-111. In one embodiment the combination treatment of the present invention is used without such additional ionizing radiation.

Radiation therapy is a standard treatment for controlling unresectable or inoperable tumors and/or tumor metastases. Improved results have been seen when radiation therapy has been combined with chemotherapy. Radiation therapy is based on the principle that high-dose radiation delivered to a target area will result in the death of reproductive cells in both tumor and normal tissues. The radiation dosage regimen is generally defined in terms of radiation absorbed dose (Gy), time and fractionation, and must be carefully defined by the oncologist. The amount of radiation a patient receives will depend on various considerations, but the two most important are the location of the tumor in relation to other critical structures or organs of the body, and the extent to which the tumor has spread. A typical course of treatment for a patient undergoing radiation therapy will be a treatment schedule over a 1 to 6 week period, with a total dose of between 10 and 80 Gy administered to the patient in a single daily fraction of about 1.8 to 2.0 Gy, 5 days a week. In a preferred embodiment of this invention there is synergy when tumors in human patients are treated with the combination treatment of the invention and radiation. In other words, the inhibition of tumor growth by means of the agents comprising the combination or single therapy of the invention is enhanced when combined with radiation, optionally with additional chemotherapeutic or anticancer agents. Parameters of adjuvant radiation therapies are, for example, contained in International Patent Publication WO 99/60023.

The antibodies are administered to a patient according to known methods, by intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, or intrathecal routes. Intravenous or subcutaneous administration of the antibodies is preferred.

The term "treating" as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, or preventing, either partially or completely, the growth of tumors, tumor metastases, or other cancer-causing or neoplastic cells in a patient. The term "treatment" as used herein, unless otherwise indicated, refers to the act of treating.

The phrase "a method of treating" or its equivalent, when applied to, for example, cancer refers to a procedure or course of action that is designed to reduce or eliminate the number of cancer cells in a patient, or to alleviate the symptoms of a cancer. "A method of treating" cancer or another proliferative disorder does not necessarily mean that the cancer cells or other disorder will, in fact, be eliminated, that the number of cells or disorder will, in fact, be reduced, or that the symptoms of a cancer or other disorder will, in fact, be alleviated. Often, a method of treating cancer will be performed even with a low likelihood of success, but which, given the medical history and estimated survival expectancy of a patient, is nevertheless deemed an overall beneficial course of action.

It is self-evident that the antibodies are administered to the patient in therapeutically effective amount which is the amount of the subject compound or combination that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

The term "first-line treatment" as used herein refers to the first type of drug therapy given for the treatment of cancer or metastasis. This can be an adjuvant or neoadjuvant or palliative chemotherapy or immunotherapy offered initially following diagnosis and/or surgery or following diagnosis of metastatic disease. The first-line treatment refers to the first cancer treatment using e.g. chemical or biochemical substances, like cytotoxic drugs, targeted therapies with antibodies, or enzyme inhibitors/antagonist/or modulators.

The present invention further provides an article of manufacture comprising a container, a composition within the container comprising an anti-HER3 antibody and a package insert instructing the user of the composition to administer said anti-HER3 antibody to a patient suffering from HER2 low, HER3 positive and HR (hormone receptor) positive (e.g. ER positive) breast cancer in combination with an anti-HER2 antibody which inhibits the dimerization of HER2.

The present invention further provides an article of manufacture comprising a container, a composition within the container comprising an anti-HER3 antibody and a package insert instructing the user of the composition to administer said anti-HER3 antibody to a patient suffering from HER2 low, HER3 positive and HR (hormone receptor) positive (e.g. ER positive) breast cancer in combination with an anti-HER2 antibody which inhibits the dimerization of HER2.

The term “package insert” refers to instructions customarily included in commercial packages of therapeutic products, which may include information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

- 5 In one embodiment, the article of manufacture containers may further include a pharmaceutically acceptable carrier. The article of manufacture may further include a sterile diluent, which is preferably stored in a separate additional container.

In the following one series of embodiments of the invention is listed:

- 10 1. An antibody which binds to human HER3 for use in the treatment of breast cancer in combination with an antibody binding to human HER2 and inhibiting dimerization of HER2, wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive.
2. The antibody according to embodiment 1, wherein the breast cancer is HER2 low HER3, and positive ER (estrogen receptor) positive.
- 15 3. The antibody according to any one of embodiments 1 to 2, wherein additionally either

A) an anti-hormonal agent is co-administered, wherein the anti-hormonal agent is selected from the group of:

- 20 a) a selective estrogen receptor degrader (SERD), (in one embodiment the estrogen receptor inhibitor is fulvestrant),
- b) a selective estrogen receptor modulator (SERM), (in one embodiment the selective estrogen receptor modulator is tamoxifen, raloxifene, toremifene or lasofoxifene), and
- 25 c) an aromatase inhibitor; (in embodiment the aromatase inhibitor is letrozole, anastrozole or exemestane);
- or

B) a chemotherapeutic agent is co-administered, wherein the chemotherapeutic agent is selected from the group of:

- 30 a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),

- 40 -

- b) cyclophosphamide (CTX),
 - c) cisplatin or carboplatin,
 - d) mitomycin C,
 - e) methotrexate (MTX),
 - 5 f) 5-fluorouracil (5-FU) or capecitabine,
 - g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo),
or daunorubicin or its liposomal formulation (daunorubicin lipo),
 - h) gemcitabine,
 - i) mitoxantrone,
 - 10 j) vinorelbine,
 - k) eribulin, and
 - l) ixabepilone.
4. The antibody according to any one of embodiments 1 to 2, wherein
additionally an anti-hormonal agent is co-administered, and wherein the anti-
15 hormonal agent is selected from the group of:
- a) a selective estrogen receptor degrader (SERD),
 - b) a selective estrogen receptor modulator (SERM); and
 - c) an aromatase inhibitor.
5. The antibody according to embodiment 4, wherein the anti-hormonal agent is
20 selected from the group of:
- a) fulvestrant,
 - b) tamoxifen, raloxifene, toremifene or lasofoxifene; and
 - c) letrozole, anastrozole or exemestane.

- 41 -

6. The antibody according to embodiment 4, wherein the anti-hormonal agent is fulvestrant.
7. The antibody according to any one of embodiments 1 to 2, wherein additionally a chemotherapeutic agent is co-administered, and wherein the chemotherapeutic agent is selected from the group of:
- a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),
 - b) cyclophosphamide (CTX),
 - c) cisplatin or carboplatin,
 - d) mitomycin C,
 - e) methotrexate (MTX),
 - f) 5-fluorouracil (5-FU) or capecitabine,
 - g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),
 - h) gemcitabine,
 - i) mitoxantrone,
 - j) vinorelbine,
 - k) eribulin, and
 - l) ixabepilone.
8. The antibody according embodiment 7, wherein the chemotherapeutic agent is a taxanes or taxane derivative selected from the group of paclitaxel, nab-paclitaxel, docetaxel (in one embodiment the chemotherapeutic agent is paclitaxel, nab-paclitaxel, in one embodiment the chemotherapeutic agent is paclitaxel).
9. The antibody according to any one of embodiments 1 to 8, wherein the combination is administered as a first-line treatment.

10. The antibody according to any one of embodiments 1 to 9, wherein the antibody which binds to human HER3 is characterized in comprising as heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.
11. The antibody according to any one of embodiments 1 to 9, wherein the antibody which binds to human HER3 is characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:10.
12. The antibody according to any one of embodiments 1 to 11, wherein the antibody which binds to human HER3 is of human IgG1 subclass is characterized in that is glycosylated with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65 % or lower.
13. The antibody according to any one of embodiments 1 to 12, wherein the antibody binding to human HER2 and inhibiting dimerization of HER2 is pertuzumab.
14. The antibody according to any one of embodiments 1 to 13, wherein the antibody which binds to human HER3 is administered at a dose of 400-600 mg every 3 weeks and the antibody which binds to human HER2 is administered at a dose of 300-500 mg every 3 weeks.

In the following another series of embodiments of the invention is listed:

1. An antibody binding to human HER2 and inhibiting dimerization of HER2 for use in the treatment of breast cancer in combination with an antibody which binds to human HER3, wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive.
2. The antibody according to embodiment 1, wherein the breast cancer is HER2 low HER3, and positive ER (estrogen receptor) positive .

3. The antibody according to any one of embodiments 1 to 2, wherein additionally either

A) an anti-hormonal agent is co-administered, wherein the anti-hormonal agent is selected from the group of:

- 5 a) a selective estrogen receptor degrader (SERD), (in one embodiment the selective estrogen receptor degrader (SERD) is fulvestrant),
- b) a selective estrogen receptor modulator (SERM), (in one embodiment the selective estrogen receptor modulator is tamoxifen, raloxifene, toremifene or lasofoxifene), and
- 10 c) an aromatase inhibitor; (in embodiment the aromatase inhibitor is letrozole, anastrozole or exemestane);
- or

B) a chemotherapeutic agent is co-administered, wherein the chemotherapeutic agent is selected from the group of:

- 15 a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),
- b) cyclophosphamide (CTX),
- c) cisplatin or carboplatin,
- d) mitomycin C,
- 20 e) methotrexate (MTX),
- f) 5-fluorouracil (5-FU) or capecitabine,
- g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),
- h) gemcitabine,
- 25 i) mitoxantrone,
- j) vinorelbine,

k) eribulin, and

l) ixabepilone.

5 4. The antibody according to any one of embodiments 1 to 2, wherein additionally an anti-hormonal agent is co-administered, and wherein the anti-hormonal agent is selected from the group of:

a) a selective estrogen receptor degrader (SERD),

b) a selective estrogen receptor modulator (SERM); and

c) an aromatase inhibitor.

10 5. The antibody according to embodiment 4, wherein the anti-hormonal agent is selected from the group of:

a) fulvestrant,

b) tamoxifen, raloxifene, toremifene or lasofoxifene; and

c) letrozole, anastrozole or exemestane.

15 6. The antibody according to embodiment 4, wherein the anti-hormonal agent is fulvestrant.

7. The antibody according to any one of embodiments 1 to 2, wherein additionally a chemotherapeutic agent is co-administered, and wherein the chemotherapeutic agent is selected from the group of:

20 a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),

b) cyclophosphamide (CTX),

c) cisplatin or carboplatin,

d) mitomycin C,

e) methotrexate (MTX),

25 f) 5-fluorouracil (5-FU) or capecitabine,

- 45 -

g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo),
or daunorubicin or its liposomal formulation (daunorubicin lipo),

h) gemcitabine,

i) mitoxantrone,

5 j) vinorelbine,

k) eribulin, and

l) ixabepilone.

8. The antibody according embodiment 7, wherein
the chemotherapeutic agent is a taxanes or taxane derivative selected from the
10 group of paclitaxel, nab-paclitaxel, docetaxel (in one embodiment the
chemotherapeutic agent is paclitaxel, nab-paclitaxel, in one embodiment the
chemotherapeutic agent is paclitaxel).

9. The antibody according to any one of embodiments 1 to 8, wherein the
combination is administered as a first-line treatment.

15 10. The antibody according to any one of embodiments 1 to 9, wherein the
antibody which binds to human HER3 is characterized in comprising as
heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H
region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the
light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a
20 CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.

11. The antibody according to any one of embodiments 1 to 9, wherein the
antibody which binds to human HER3 is characterized in that

the heavy chain variable domain VH is SEQ ID NO:8; and the light chain
variable domain VL is SEQ ID NO:10.

25 12. The antibody according to any one of embodiments 1 to 11, wherein the
antibody which binds to human HER3 is of human IgG1 subclass and is
characterized in that the antibody is glycosylated with a sugar chain at
Asn297 whereby the amount of fucose within said sugar chain is 65 % or
lower.

- 46 -

13. The antibody according to any one of embodiments 1 to 12, wherein the antibody binding to human HER2 and inhibiting dimerization of HER2 is pertuzumab.
- 5 14. The antibody according to any one of embodiments 1 to 13, wherein the antibody which binds to human HER3 is administered at a dose of 400-600 mg every 3 weeks and the antibody which binds to human HER2 is administered at a dose of 300-500 mg every 3 weeks.

In the following another series of embodiments of the invention is listed:

- 10 1. An antibody which binds to human HER3 and/ or an antibody binding to human HER2 and inhibiting dimerization of HER2, for use in the manufacture of a medicament for the combination treatment of breast cancer wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive.
- 15 2. The use according to embodiment 1, wherein the breast cancer is HER2 low HER3, and positive ER (estrogen receptor) positive .
3. The use according to any one of embodiments 1 to 2, wherein additionally either
- A) an anti-hormonal agent is co-administered, wherein the anti-hormonal agent is selected from the group of:
- 20 a) a selective estrogen receptor degrader (SERD), (in one embodiment the selective estrogen receptor degrader (SERD) is fulvestrant),
- b) a selective estrogen receptor modulator (SERM), (in one embodiment the selective estrogen receptor modulator is tamoxifen, raloxifene, toremifene or lasofoxifene), and
- 25 c) an aromatase inhibitor; (in embodiment the aromatase inhibitor is letrozole, anastrozole or exemestane);
- or

B) a chemotherapeutic agent is co-administered, wherein the chemotherapeutic agent is selected from the group of:

- a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),
- 5 b) cyclophosphamide (CTX),
- c) cisplatin or carboplatin,
- d) mitomycin C,
- e) methotrexate (MTX),
- f) 5-fluorouracil (5-FU) or capecitabine,
- 10 g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),
- h) gemcitabine,
- i) mitoxantrone,
- j) vinorelbine,
- 15 k) eribulin, and
- l) ixabepilone.

4. The use according to any one of embodiments 1 to 2, wherein additionally an anti-hormonal agent is co-administered, and wherein the anti-hormonal agent is selected from the group of:

- 20 a) a selective estrogen receptor degrader (SERD),
- b) a selective estrogen receptor modulator (SERM); and
- c) an aromatase inhibitor.

5. The use according to embodiment 4, wherein the anti-hormonal agent is selected from the group of:
- a) fulvestrant,
 - b) tamoxifen, raloxifene, toremifene or lasofoxifene; and
 - c) letrozole, anastrozole or exemestane.
6. The use according to embodiment 4, wherein the anti-hormonal agent is fulvestrant.
7. The use according to any one of embodiments 1 to 2, wherein additionally a chemotherapeutic agent is co-administered, and wherein the chemotherapeutic agent is selected from the group of:
- a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),
 - b) cyclophosphamide (CTX),
 - c) cisplatin or carboplatin,
 - d) mitomycin C,
 - e) methotrexate (MTX),
 - f) 5-fluorouracil (5-FU) or capecitabine,
 - g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),
 - h) gemcitabine,
 - i) mitoxantrone,
 - j) vinorelbine,
 - k) eribulin, and
 - l) ixabepilone.

8. The use according embodiment 7, wherein
the chemotherapeutic agent is a taxanes or taxane derivative selected from the
group of paclitaxel, nab-paclitaxel, docetaxel (in one embodiment the
chemotherapeutic agent is paclitaxel, nab-paclitaxel, in one embodiment the
chemotherapeutic agent is paclitaxel).
9. The use according to any one of embodiments 1 to 8, wherein the
combination is administered as a first-line treatment.
10. The use according to any one of embodiments 1 to 9, wherein the antibody
which binds to human HER3 is characterized in comprising as heavy chain
variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of
SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain
variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L
region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.
11. The use according to any one of embodiments 1 to 9, wherein the antibody
which binds to human HER3 is characterized in that
the heavy chain variable domain VH is SEQ ID NO:8; and the light chain
variable domain VL is SEQ ID NO:10.
12. The use according to any one of embodiments 1 to 11, wherein the antibody
which binds to human HER3 is of human IgG1 subclass and is characterized
in that the antibody is glycosylated with a sugar chain at Asn297 whereby the
amount of fucose within said sugar chain is 65 % or lower.
13. The use according to any one of embodiments 1 to 12, wherein the antibody
binding to human HER2 and inhibiting dimerization of HER2 is pertuzumab.
14. The use according to any one of embodiments 1 to 13, wherein the antibody
which binds to human HER3 is administered at a dose of 400-600 mg every 3
weeks and the antibody which binds to human HER2 is administered at a
dose of 300-500 mg every 3 weeks.

In the following another series of embodiments of the invention is listed:

1. The use of antibody which binds to human HER3 and/ or an antibody binding
to human HER2 and inhibiting dimerization of HER2, for the manufacture
of a medicament for the combination treatment of breast cancer wherein the

breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive.

2. The use according to embodiment 1, wherein the breast cancer is HER2 low HER3, and positive ER (estrogen receptor) positive .

5 3. The use according to any one of embodiments 1 to 2, wherein additionally either

A) an anti-hormonal agent is co-administered, wherein the anti-hormonal agent is selected from the group of:

10 a) a selective estrogen receptor degrader (SERD), (in one embodiment the selective estrogen receptor degrader (SERD) is fulvestrant),

b) a selective estrogen receptor modulator (SERM), (in one embodiment the selective estrogen receptor modulator is tamoxifen, raloxifene, toremifene or lasofoxifene), and

15 c) an aromatase inhibitor; (in embodiment the aromatase inhibitor is letrozole, anastrozole or exemestane);
or

B) a chemotherapeutic agent is co-administered, wherein the chemotherapeutic agent is selected from the group of:

20 a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),

b) cyclophosphamide (CTX),

c) cisplatin or carboplatin,

d) mitomycin C,

e) methotrexate (MTX),

25 f) 5-fluorouracil (5-FU) or capecitabine,

g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),

- 51 -

h) gemcitabine,

i) mitoxantrone,

j) vinorelbine,

k) eribulin, and

5 l) ixabepilone.

4. The use according to any one of embodiments 1 to 2, wherein additionally an anti-hormonal agent is co-administered, and wherein the anti-hormonal agent is selected from the group of:

a) a selective estrogen receptor degrader (SERD),

10 b) a selective estrogen receptor modulator (SERM); and

c) an aromatase inhibitor.

5. The use according to embodiment 4, wherein the anti-hormonal agent is selected from the group of:

a) fulvestrant,

15 b) tamoxifen, raloxifene, toremifene or lasofoxifene; and

c) letrozole, anastrozole or exemestane.

6. The use according to embodiment 4, wherein the anti-hormonal agent is fulvestrant.

7. The use according to any one of embodiments 1 to 2, wherein additionally a chemotherapeutic agent is co-administered, and wherein the chemotherapeutic agent is selected from the group of:

a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),

b) cyclophosphamide (CTX),

25 c) cisplatin or carboplatin,

d) mitomycin C,

- 52 -

e) methotrexate (MTX),

f) 5-fluorouracil (5-FU) or capecitabine,

g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo),
or daunorubicin or its liposomal formulation (daunorubicin lipo),

5 h) gemcitabine,

i) mitoxantrone,

j) vinorelbine,

k) eribulin, and

l) ixabepilone.

10 8. The use according embodiment 7, wherein
the chemotherapeutic agent is a taxanes or taxane derivative selected from the
group of paclitaxel, nab-paclitaxel, docetaxel (in one embodiment the
chemotherapeutic agent is paclitaxel, nab-paclitaxel, in one embodiment the
chemotherapeutic agent is paclitaxel).

15 9. The use according to any one of embodiments 1 to 8, wherein the
combination is administered as a first-line treatment.

10. The use according to any one of embodiments 1 to 9, wherein the antibody
which binds to human HER3 is characterized in comprising as heavy chain
variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of
20 SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain
variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L
region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.

11. The use according to any one of embodiments 1 to 9, wherein the antibody
which binds to human HER3 is characterized in that

25 the heavy chain variable domain VH is SEQ ID NO:8; and the light chain
variable domain VL is SEQ ID NO:10.

12. The use according to any one of embodiments 1 to 11, wherein the antibody
which binds to human HER3 is of human IgG1 subclass and is characterized

in that the antibody is glycosylated with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65 % or lower.

13. The use according to any one of embodiments 1 to 12, wherein the antibody binding to human HER2 and inhibiting dimerization of HER2 is pertuzumab.
- 5 14. The use according to any one of embodiments 1 to 13, wherein the antibody which binds to human HER3 is administered at a dose of 400-600 mg every 3 weeks and the antibody which binds to human HER2 is administered at a dose of 300-500 mg every 3 weeks.

In the following another series of embodiments of the invention is listed:

- 10 1. The use of antibody which binds to human HER3 for the manufacture of a medicament for the treatment of breast cancer wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive, and wherein the treatment is in combination with an antibody binding to human HER2 and inhibiting dimerization of HER2.
- 15 2. The use according to embodiment 1, wherein the breast cancer is HER2 low HER3, and positive ER (estrogen receptor) positive .
3. The use according to any one of embodiments 1 to 2, wherein additionally either
- 20 A) an anti-hormonal agent is co-administered, wherein the anti-hormonal agent is selected from the group of:
- a) a selective estrogen receptor degrader (SERD), (in one embodiment the selective estrogen receptor degrader (SERD) is fulvestrant),
- b) a selective estrogen receptor modulator (SERM), (in one embodiment the selective estrogen receptor modulator is tamoxifen, raloxifene, toremifene or lasofoxifene), and
- 25 c) an aromatase inhibitor; (in embodiment the aromatase inhibitor is letrozole, anastrozole or exemestane);
- or

B) a chemotherapeutic agent is co-administered, wherein the chemotherapeutic agent is selected from the group of:

- a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),
- 5 b) cyclophosphamide (CTX),
- c) cisplatin or carboplatin,
- d) mitomycin C,
- e) methotrexate (MTX),
- f) 5-fluorouracil (5-FU) or capecitabine,
- 10 g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),
- h) gemcitabine,
- i) mitoxantrone,
- j) vinorelbine,
- 15 k) eribulin, and
- l) ixabepilone.

4. The use according to any one of embodiments 1 to 2, wherein additionally an anti-hormonal agent is co-administered, and wherein the anti-hormonal agent is selected from the group of:

- 20 a) a selective estrogen receptor degrader (SERD),
- b) a selective estrogen receptor modulator (SERM); and
- c) an aromatase inhibitor.

5. The use according to embodiment 4, wherein the anti-hormonal agent is selected from the group of:

- 25 a) fulvestrant,

- 55 -

- b) tamoxifen, raloxifene, toremifene or lasofoxifene; and
- c) letrozole, anastrozole or exemestane.

6. The use according to embodiment 4, wherein the anti-hormonal agent is fulvestrant.

5 7. The use according to any one of embodiments 1 to 2, wherein additionally a chemotherapeutic agent is co-administered, and wherein the chemotherapeutic agent is selected from the group of:

a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),

10 b) cyclophosphamide (CTX),

c) cisplatin or carboplatin,

d) mitomycin C,

e) methotrexate (MTX),

f) 5-fluorouracil (5-FU) or capecitabine,

15 g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),

h) gemcitabine,

i) mitoxantrone,

j) vinorelbine,

20 k) eribulin, and

l) ixabepilone.

8. The use according embodiment 7, wherein the chemotherapeutic agent is a taxanes or taxane derivative selected from the group of paclitaxel, nab-paclitaxel, docetaxel (in one embodiment the chemotherapeutic agent is paclitaxel, nab-paclitaxel, in one embodiment the chemotherapeutic agent is paclitaxel).

25

- 56 -

9. The use according to any one of embodiments 1 to 8, wherein the combination is administered as a first-line treatment.
10. The use according to any one of embodiments 1 to 9, wherein the antibody which binds to human HER3 is characterized in comprising as heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.
11. The use according to any one of embodiments 1 to 9, wherein the antibody which binds to human HER3 is characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:10.
12. The use according to any one of embodiments 1 to 11, wherein the antibody which binds to human HER3 is of human IgG1 subclass and is characterized in that the antibody is glycosylated with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65 % or lower.
13. The use according to any one of embodiments 1 to 12, wherein the antibody binding to human HER2 and inhibiting dimerization of HER2 is pertuzumab.
14. The use according to any one of embodiments 1 to 13, wherein the antibody which binds to human HER3 is administered at a dose of 400-600 mg every 3 weeks and the antibody which binds to human HER2 is administered at a dose of 300-500 mg every 3 weeks.

In the following another series of embodiments of the invention is listed:

1. The use of an antibody binding to human HER2 and inhibiting dimerization of HER2, for the manufacture of a medicament for the treatment of breast cancer wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive and wherein the treatment is in combination with an antibody which binds to human HER3.
2. The use according to embodiment 1, wherein the breast cancer is HER2 low HER3, and positive ER (estrogen receptor) positive .

3. The use according to any one of embodiments 1 to 2, wherein additionally either

A) an anti-hormonal agent is co-administered, wherein the anti-hormonal agent is selected from the group of:

- 5 a) a selective estrogen receptor degrader (SERD), (in one embodiment the selective estrogen receptor degrader (SERD) is fulvestrant),
- b) a selective estrogen receptor modulator (SERM), (in one embodiment the selective estrogen receptor modulator is tamoxifen, raloxifene, toremifene or lasofoxifene), and
- 10 c) an aromatase inhibitor; (in embodiment the aromatase inhibitor is letrozole, anastrozole or exemestane);
- or

B) a chemotherapeutic agent is co-administered, wherein the chemotherapeutic agent is selected from the group of:

- 15 a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),
- b) cyclophosphamide (CTX),
- c) cisplatin or carboplatin,
- d) mitomycin C,
- 20 e) methotrexate (MTX),
- f) 5-fluorouracil (5-FU) or capecitabine,
- g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),
- h) gemcitabine,
- 25 i) mitoxantrone,
- j) vinorelbine,

k) eribulin, and

l) ixabepilone.

4. The use according to any one of embodiments 1 to 2, wherein additionally an anti-hormonal agent is co-administered, and wherein the anti-hormonal agent is selected from the group of:
- 5
- a) a selective estrogen receptor degrader (SERD),
 - b) a selective estrogen receptor modulator (SERM); and
 - c) an aromatase inhibitor.
5. The use according to embodiment 4, wherein the anti-hormonal agent is selected from the group of:
- 10
- a) fulvestrant,
 - b) tamoxifen, raloxifene, toremifene or lasofoxifene; and
 - c) letrozole, anastrozole or exemestane.
6. The use according to embodiment 4, wherein the anti-hormonal agent is fulvestrant.
- 15
7. The use according to any one of embodiments 1 to 2, wherein additionally a chemotherapeutic agent is co-administered, and wherein the chemotherapeutic agent is selected from the group of:
- a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),
 - b) cyclophosphamide (CTX),
 - c) cisplatin or carboplatin,
 - d) mitomycin C,
 - e) methotrexate (MTX),
 - f) 5-fluorouracil (5-FU) or capecitabine,
- 20
- 25

- 59 -

g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo),
or daunorubicin or its liposomal formulation (daunorubicin lipo),

h) gemcitabine,

i) mitoxantrone,

5 j) vinorelbine,

k) eribulin, and

l) ixabepilone.

8. The use according embodiment 7, wherein
the chemotherapeutic agent is a taxanes or taxane derivative selected from the
10 group of paclitaxel, nab-paclitaxel, docetaxel (in one embodiment the
chemotherapeutic agent is paclitaxel, nab-paclitaxel, in one embodiment the
chemotherapeutic agent is paclitaxel).

9. The use according to any one of embodiments 1 to 8, wherein the
combination is administered as a first-line treatment.

15 10. The use according to any one of embodiments 1 to 9, wherein the antibody
which binds to human HER3 is characterized in comprising as heavy chain
variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of
SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain
variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L
20 region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.

11. The use according to any one of embodiments 1 to 9, wherein the antibody
which binds to human HER3 is characterized in that

the heavy chain variable domain VH is SEQ ID NO:8; and the light chain
variable domain VL is SEQ ID NO:10.

25 12. The use according to any one of embodiments 1 to 11, wherein the antibody
which binds to human HER3 is of human IgG1 subclass and is characterized
in that the antibody is glycosylated with a sugar chain at Asn297 whereby the
amount of fucose within said sugar chain is 65 % or lower.

- 60 -

13. The use according to any one of embodiments 1 to 12, wherein the antibody binding to human HER2 and inhibiting dimerization of HER2 is pertuzumab.

14. The use according to any one of embodiments 1 to 13, wherein the antibody which binds to human HER3 is administered at a dose of 400-600 mg every 3 weeks and the antibody which binds to human HER2 is administered at a dose of 300-500 mg every 3 weeks.

In the following another series of embodiments of the invention is listed:

1. A method of treating a patient suffering from breast cancer wherein the breast cancer is HER2 low, HER3 positive and HR (hormone receptor) positive, the method comprising the administration of (an effective amount of) an antibody which binds to human HER3 and the co-administration of (an effective amount of) an antibody binding to human HER2 and inhibiting dimerization of HER2 .

2. The method according to embodiment 1, wherein the breast cancer is HER2 low HER3, and positive ER (estrogen receptor) positive .

3. The method according to any one of embodiments 1 to 2, wherein additionally either

A) an anti-hormonal agent is co-administered, wherein the anti-hormonal agent is selected from the group of:

a) a selective estrogen receptor degrader (SERD), (in one embodiment the selective estrogen receptor degrader (SERD) is fulvestrant),

b) a selective estrogen receptor modulator (SERM), (in one embodiment the selective estrogen receptor modulator is tamoxifen, raloxifene, toremifene or lasofoxifene), and

c) an aromatase inhibitor; (in embodiment the aromatase inhibitor is letrozole, anastrozole or exemestane);
or

B) a chemotherapeutic agent is co-administered, wherein the chemotherapeutic agent is selected from the group of:

- a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),
- 5 b) cyclophosphamide (CTX),
- c) cisplatin or carboplatin,
- d) mitomycin C,
- e) methotrexate (MTX),
- f) 5-fluorouracil (5-FU) or capecitabine,
- 10 g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),
- h) gemcitabine,
- i) mitoxantrone,
- j) vinorelbine,
- 15 k) eribulin, and
- l) ixabepilone.

4. The method according to any one of embodiments 1 to 2, wherein additionally an anti-hormonal agent is co-administered, and wherein the anti-hormonal agent is selected from the group of:

- 20 a) a selective estrogen receptor degrader (SERD),
- b) a selective estrogen receptor modulator (SERM); and
- c) an aromatase inhibitor.

5. The method according to embodiment 4, wherein the anti-hormonal agent is selected from the group of:

- 25 a) fulvestrant,

- 62 -

- b) tamoxifen, raloxifene, toremifene or lasofoxifene; and
- c) letrozole, anastrozole or exemestane.

6. The method according to embodiment 4, wherein the anti-hormonal agent is fulvestrant.

5 7. The method according to any one of embodiments 1 to 2, wherein additionally a chemotherapeutic agent is co-administered, and wherein the chemotherapeutic agent is selected from the group of:

a) a taxane or taxane derivative (like paclitaxel and paclitaxel derivatives, or docetaxel),

10 b) cyclophosphamide (CTX),

c) cisplatin or carboplatin,

d) mitomycin C,

e) methotrexate (MTX),

f) 5-fluorouracil (5-FU) or capecitabine,

15 g) doxorubicin (DXR) or its liposomal formulation (doxorubicin lipo), or daunorubicin or its liposomal formulation (daunorubicin lipo),

h) gemcitabine,

i) mitoxantrone,

j) vinorelbine,

20 k) eribulin, and

l) ixabepilone.

8. The method according embodiment 7, wherein the chemotherapeutic agent is a taxanes or taxane derivative selected from the group of paclitaxel, nab-paclitaxel, docetaxel (in one embodiment the chemotherapeutic agent is paclitaxel, nab-paclitaxel, in one embodiment the chemotherapeutic agent is paclitaxel).

25

9. The method according to any one of embodiments 1 to 8, wherein the combination is administered as a first-line treatment.
10. The method according to any one of embodiments 1 to 9, wherein the antibody which binds to human HER3 is characterized in comprising as
5 heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.
11. The method according to any one of embodiments 1 to 9, wherein the
10 antibody which binds to human HER3 is characterized in that

the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:10.
12. The method according to any one of embodiments 1 to 11, wherein the
15 antibody which binds to human HER3 is of human IgG1 subclass and is characterized in that the antibody is glycosylated with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65 % or lower.
13. The method according to any one of embodiments 1 to 12, wherein the
20 antibody binding to human HER2 and inhibiting dimerization of HER2 is pertuzumab.
14. The method according to any one of embodiments 1 to 13, wherein the antibody which binds to human HER3 is administered at a dose of 400-600 mg every 3 weeks and the antibody which binds to human HER2 is administered at a dose of 300-500 mg every 3 weeks.
- 25 The following examples, sequence listing and figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Description of the Sequence Listing

- | | | |
|----|--------------|-------------------------------|
| 30 | SEQ ID NO: 1 | heavy chain CDR3H, Mab 205.10 |
| | SEQ ID NO: 2 | heavy chain CDR2H, Mab 205.10 |

- 64 -

	SEQ ID NO: 3	heavy chain CDR1H, Mab 205.10
	SEQ ID NO: 4	light chain CDR3L, Mab 205.10
	SEQ ID NO: 5	light chain CDR2L, Mab 205.10
	SEQ ID NO: 6	light chain CDR1L (variant 1), Mab 205.10
5	SEQ ID NO: 7	light chain CDR1L (variant 2), Mab 205.10
	SEQ ID NO: 8	heavy chain variable domain VH, Mab 205.10
	SEQ ID NO: 9	light chain variable domain VL, Mab 205.10.1
	SEQ ID NO: 10	light chain variable domain VL, Mab 205.10.2
	SEQ ID NO: 11	light chain variable domain VL, Mab 205.10.3
10	SEQ ID NO: 12	human kappa light chain constant region
	SEQ ID NO: 13	human heavy chain constant region derived from IgG1
	SEQ ID NO: 14	human heavy chain constant region derived from IgG1 mutated on L234A and L235A
	SEQ ID NO: 15	human heavy chain constant region derived from IgG4
15	SEQ ID NO: 16	human heavy chain constant region derived from IgG4 mutated on S228P
	SEQ ID NO: 17	human HER3 (including signal peptide)
	SEQ ID NO: 18	human HER2 (including signal peptide)
	SEQ ID NO: 19	human ER (including signal peptide)
20	SEQ ID NO: 20	heavy chain variable domain VH, pertuzumab
	SEQ ID NO: 21	light chain variable domain VL, pertuzumab

Examples

Example 1

25 Immunisation

NMRI mice were immunized with hHER3-ECD (in-house) and boosted with hu-HER3-ECD. The immune response was monitored by testing serum samples against the HER1/2/3- ECD-ELISA. Spleen cells from mice with sufficient titers of anti-HER3 immunoglobulin were frozen for later immortalization by fusion with

30 mouse myeloma cell line P3X63 Ag8.653. One fusion was done and hybridoma supernatants screened by HER1/2/-ECD- ELISA showing no cross-reactivity, but binding to HER3-ECD and anti-HER3 selective hybridomas were selected. The relevant hybridomas were cloned by single cell FACS sorting. Single cell clones from different hybridomas were cultured in vitro to produce antibody in tissue

35 culture medium for characterization. Antibodies were selected by determining their ability to inhibit HER3 phosphorylation, AKT phosphorylation and tumor cell

proliferation of MDA-MB-175 cells (see Examples below). From the obtained antibodies, one was further humanized to give the following antibodies Mab 205.10.1, Mab 205.10.2 and Mab 205.10.3 with their respective VH and VL or CDRs.

Antibody	VH	VL
Mab 205.10.1	SEQ ID NO: 8	SEQ ID NO: 9
Mab 205.10.2	SEQ ID NO: 8	SEQ ID NO: 10
Mab 205.10.3	SEQ ID NO: 8	SEQ ID NO: 11

5

Antibody	CDR3H	CDR2H	CDR1H	CDR3L	CDR2L	CDR1L
Mab 205.10.1	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 6
Mab 205.10.2	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 7
Mab 205.10.3	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 6

In one embodiment such antibodies were prepared using constant regions of human origin e.g. SEQ ID NO:12-13.

Example 2

10

Binding assays

a) Antigen specific ELISA for binding to human HER3 ECD

15

Soluble human HER3 extracellular domain fused to Streptavidin Binding Protein (SBP) was captured on a streptavidine plate. To define optimal binding of the antibody to SPB-CDCP1, 384-well polystyrene plates (NUNC, streptavidin-coated) delivered by MicroCoat, Bernried, Germany (ID-No.1734776-001) have been coated with pure and stepwise diluted HEK293 supernatant (in BSA/IMDM buffer:100 mg/ml BSA Fraction V, Roche 10735078001, dissolved in Iscove's Modified Dulbeccos Medium). Using mouse a calibration curve of chimeric 205 antibodies the optimal dilution factor of the HEK293 supernatant in relation to the streptavidin binding capacity of the microtiter plate was identified. For the standard coating, SBP-HER3 containing HEK293 supernatant was diluted (between 1:15 and 1: 40) and incubated overnight at 2-80C (25µl per well). Intensive washing of the microtiter plate is necessary to remove remaining unbound SBP-HER3.

20

Antibodies according to the invention antibody were tested either undiluted or using a 12-step-dilution. 12.5 µl per well of each sample was incubated for 90 min at room temperature. After intensive washing using PBS-T (0.1% Tween 20 in PBS) 25 µl goat anti-human IgG antibodies coupled with HRP (Jackson ImmunoResearch, Code No: 109-036-098, dilution 1:10000) for human antibodies were added and incubated for 1 hour. After intensive washing the binding of the antibodies was detected with ABTS tablets (Roche Diagnostics GmbH, Cat.No.: 1112422). Absorbance at 405 nm/492 nm was measured using a standard photometer.

The table shows the relative binding ratios of the different antibodies.

antibody	hu_HER3-ECD-ELISA c(µg/ml)	IgG-ELISA c(µg/ml)	activity (ratio binding to hu_HER3-ECD/IgG)
Mab 205.10.1	583.,1	785.,0	0.74
Mab 205.10.2	396.,4	508.,0	0.,78
Mab 205.10.3	505.4	608.4	0.83

b) Characterization of the binding of anti-HER3 antibodies to a extracellular-domain-(ECD) fragment of human HER3 by Biacore analyses:

For affinity measurements, 30 µg/ml of anti Fcγ antibodies (from goat, Jackson Immuno Research) were coupled to the surface of a CM-5 sensor chip by standard amine-coupling and blocking chemistry on a SPR instrument (Biacore T100). After conjugation, anti-HER3 antibodies were injected at 25°C at a flow rate of 5 µL/min, followed by a dilution series (0 nM to 1000 nM) of human HER3 ECD at 30 µL/min. As running buffer for the binding experiment PBS/0.1% BSA was used. The chip was then regenerated with a 60s pulse of 10 mM glycine-HCl, pH 2.0 solution.

Calculation of thermodynamic parameters (K_D , binding constant to HER3) were calculated using a Langmuir 1:1 binding model.

Antibody	Binding Affinity KD [M]
Mab 205.10.1	2.0×10^{-9}
Mab 205.10.2	1.1×10^{-9}
Mab 205.10.3	2.0×10^{-9}

In a competitive binding assay (Biacore) Mab205.10.1, Mab205.10.2, and Mab205.10.3 all showed binding to the same epitope. The anti-HER3-antibodies U1-7, U-53 and U1-59 described in WO 2007/077028 and Ab#6 described in WO 2008/100624 were investigated in such assay and revealed to bind to different epitopes than antibodies Mab205.10.1, Mab205.10.2, and Mab205.10.3.

Example 3

a) Inhibition of HER3 phosphorylation in MCF7, FaDu and Mel-Juso cells

Assays were performed in MCF7 and FaDu cells according to the following protocol: Seed cells with 500,000 cells/well into Poly-D-Lysine coated 6-well plate in RPMI1640 medium with 10% FCS. Incubate for 24h. Remove medium by aspirating, incubate overnight with 500µl/well RPMI 1640 with 0.5% FCS. Add antibodies in 500 µl RPMI 1640 with 0.5% FCS. Incubate for 1h. Add HRG-1b (final concentration 500ng/ml) for 10 min. To lyse the cells remove medium and add 80 µl ice cold Triton-X-100 cell lysis buffer and incubate for 5 minutes on ice. After transferring the lysate into 1.5 ml reaction tube and centrifugation at 14000 rpm for 15 min at 4°C, transfer supernatant into fresh reaction tubes. Samples containing equal amounts of protein in SDS loading buffer were separated on SDS PAGE and blotted by using a semi-dry Western Blot to nitrocellulose membranes. Membranes were blocked by 1xNET-buffer + 0.25% gelatine for 1h hour and pHER3 is detected by the antibody αPhospho-HER3/ErbB3 (Tyr1289)(21D3), Cell Signaling, #4791 and HER3 by the antibody αErbB3 (C-17), Santa Cruz, #sc-285 respectively. After washing und detection of the signals by an POD coupled secondary antibody, bands were densometrically scanned. The anti-HER3 antibodies Mab205.10.1, Mab205.10.2, and Mab205.10.3 and also anti-HER3 antibodies U1-7, U-53 and U1-59 described in WO 2007/077028 and Ab#6 described in WO 2008/100624 were investigated. Percent (%) inhibition of anti-HER3 antibodies on receptor phosphorylation in MCF7 cells is shown below and in Fig 1A.

% Inhibition of HER3 phosphorylation in MCF7 cells

Antibody	pHER3 % inhibition [0.1 µg/ml]	pHER3 % inhibition [1.0 µg/ml]
control	0	0
Mab205.10.2	62	96
U1-7	36	44
U1-53	54	51
U1-59	15	70
Ab#6	13	64

5 In a further experiment the anti-HER3 antibody Mab205.10.2, and also the anti-HER3-antibodies 8B8.2D9 described in WO 97/35885, and 1B4C3 and 2D1D12 described in WO 2003/013602 were investigated. Percent (%) inhibition of anti-HER3 antibodies on receptor phosphorylation in MCF7 cells is shown below and in Fig 1B.

% Inhibition of HER3 phosphorylation in MCF7 cells

Antibody	pHER3 % inhibition [0.1 µg/ml]	pHER3 % inhibition [1.0 µg/ml]
control	0	0
Mab205.10.2	68	91
8B8.2D9	13	28
1B4C3	49	46
2D1D12	34	34

10 Percent (%) inhibition of anti-HER3 antibodies on receptor phosphorylation in FaDu cells is shown below.

% Inhibition of HER3 phosphorylation in FaDu cells

Antibody	pHER3 % Inhibition [0.03 µg/ml]	pHER3 % Inhibition [0.10 µg/ml]	pHER3 % Inhibition [0.30 µg/ml]
Control	0	0	0
Mab205.10.2	88	93	97
U1-59	31	25	90

In a further experiment, the anti-HER3 antibody Mab205.10.2, and also the anti-HER3-antibodies 8B8.2D9 described in WO 97/35885, and 1B4C3 and 2D1D12 described in WO 2003/013602, and 105.5 from (Millipore, Cat.no. 05-47, named α -HER^{ECD} in WO 2003/013602) were investigated in Mel-Juso cells. Assays in Mel-Juso cells were performed according to the aforementioned protocol for MCF7 and FaDu cells. Cell numbers and media volumes were adapted to 12-well plates. Percent (%) inhibition of anti-HER3 antibodies on receptor phosphorylation in Mel-Juso cells is shown below and in Figure 1C.

% Inhibition of HER3 phosphorylation in Mel-Juso cells

Antibody	pHER3 % inhibition [0.1 µg/ml]	pHER3 % inhibition [1.0 µg/ml]
control	0	0
Mab205.10.2	75.9	78.8
105.5 (α -HER ^{ECD})	22.2	19.5
8B8.2D9	31.3	20.3
1B4C3	20.7	17.5
2D1D12	3.4	39.3

b) AKT phosphorylation (ELISA)

Assays were performed in MCF7 cells according to the following protocol: Seed MCF7 cells with 30000 cells/well into Poly-D-Lysine coated 96-well plate in RPMI1640 medium with 10% FCS and incubate for 24h. Remove medium by tapping on a clean paper towel, wash carefully with 200µl serum-free medium, incubate overnight with 100µl/well RPMI 1640 with 0.5% FCS. Remove medium as above; add antibodies in 100µl RPMI 1640 with 0.5% FCS and incubate 1.5h. Add HRG-1b (final concentration 5ng/ml) for 10 min. Remove medium as above. To lyse the cells add 100µl ice cold cell lysis buffer on ice and resuspended by

pipetting ca.5x. Centrifuge plate at 3000rpm for 10min at 4°C and transfer 80µl supernatant (or aliquots) into fresh polypropylene plate and shock-freeze in LN2. Store at -80°C until assay.

5 AKT1,2(phospho-Ser473) EIA Kit Assay Designs #900-162:Samples (1:10 diluted) are added to the plate coated with a mouse monoclonal antibody specific for the N-terminus of AKT. Incubation 1h at RT with shaking. Wash 5x, incubation with biotinylated anti-phospho-AKT(Ser473) 1h at RT with shaking. Wash 5x, incubation with streptavidin-HRP conjugate 30min at RT with shaking. Wash 5x, incubate with TMB substrate 30min at RT with shaking. Stop and read at 450nm.

10 Mab 205.10.2 showed an IC₅₀ of the AKT phosphorylation inhibition of 0.06 µg/ml.

In an pAKT ELISA in Mel-Juso cell performed as described for MCF7 cells Mab 205.10.2 showed an IC₅₀ of AKT phosphorylation inhibition of 0.28 µg/ml all the other analyses antibodies show an IC₅₀ above (>) 50.

15 **% AKT phosphorylation inhibition in Mel-Juso cells**

Antibody	IC ₅₀ [µg/ml]
Mab 205.10.2	0.28
105.5 (α-HER ^{ECD})	0.81
1B4C3	>50
2D1D12	>50
8B8D9	>50

c) Inhibition of tumor cell proliferation

20 The anti-tumor efficacy of HER3 antibodies Mab205.10.1, Mab205.10.2, and Mab205.10.3 in a cell proliferation assay, using MDA-MB-175 cells (VII Human Breast Carcinoma Cells, ATCC catalog no. HTB-25), was assessed. 20,000 cells per well were seeded into sterile 96 well tissue culture plates with DMEM/F12 cell culture medium, containing 10% FCS and incubated at 37°C±1°C with 5 % ±1% CO₂ for one day. The cells are slow growing cells with a doubling time of ca. 1.5 days. Anti-HER3 antibodies were added in dilution series and further incubated for

25 6 days. Cell viability was then assessed using the alamarBlue® readout. If the cell viability was reduced to more than 50 % of control, IC₅₀ values were calculated using means of triplicates for each antibody concentration; otherwise, if the %

inhibition of cell viability at the highest concentration was below 50%, no IC₅₀ could be calculated and it is indicated that IC₅₀ [µg/ml] is above (>) the highest concentration. Also the anti-HER3-antibodies U1-59 described in WO 2007/077028 and Ab#6 described in WO 2008/100624 were investigated.

antibody	IC ₅₀ [µg/ml]
Mab205.10.1	8.0
Mab205.10.2	3.8
Mab205.10.3	6.8
U1-59	12.4
Ab#6	> 60 µg/ml

5

In a further experiment the anti-HER3 antibodies 8B8.2D9 described in WO 97/35885, and 1B4C3 described in WO 2003/013602 were investigated.

antibody	IC ₅₀ [µg/ml]
8B8.2D9	> 100 µg/ml (29 % inhibition at 100 µg/ml)
1B4C3	> 100 µg/ml (26 % inhibition at 100 µg/ml)

10 **Example 4**

In vitro ADCC in KPL-4 tumor cells by 1µg/ml specific Lysis %

The target cells KPL4 (ADCC), breast carcinoma, cultivation in RPMI1640 + 2 mM L-alanyl-L-Glutamine + 10 % FCS) were collected with trypsin/EDTA (Gibco # 25300-054) in exponential growth phase. After a washing step and checking cell number and viability, the aliquot needed was labeled for 30 min at 37°C in the cell incubator with calcein (Invitrogen #C3100MP; 1 vial was resuspended in 50 µl DMSO for 5 Mio cells in 5 ml medium). Afterwards, the cells were washed three times with AIM-V medium, the cell number and viability was checked and the cell number adjusted to 0.3 Mio/ml.

20 Meanwhile, PBMC (Peripheral Blood Mononuclear Cells) as effector cells were prepared by density gradient centrifugation (Histopaque-1077, Sigma # H8889) according to the manufacturer's protocol (washing steps 1x at 400g and 2x at 350g

10 min each). The cell number and viability was checked and the cell number adjusted to 15 Mio/ml.

100µl calcein-stained target cells were plated in round-bottom 96-well plates, 50µl diluted, afucosylated antibody (Mab205.10.1, Mab205.10.2, Mab205.10.3, preparation see below) which was added and 50µl effector cells. In some experiments the target cells were mixed with Redimune® NF Liquid (ZLB Behring) at a concentration of 10 mg/ml Redimune.

As controls served the spontaneous lysis, determined by co-culturing target and effector cells without antibody and the maximal lysis, determined by 1 % Triton X-100 lysis of target cells only. The plate was incubated for 4 hours at 37°C in a humidified cell incubator.

The killing of target cells was assessed by measuring LDH (Lactate Dehydrogenase) release from damaged cells using the Cytotoxicity Detection kit (LDH Detection Kit, Roche # 1 644 793) according to the manufacturer's instruction. Briefly, 100 µl supernatant from each well was mixed with 100 µl substrate from the kit in a transparent flat bottom 96 well plate. The Vmax values of the substrate's colour reaction was determined in an ELISA reader at 490 nm for at least 10 min. Percentage of specific antibody-mediated killing was calculated as follows: $(A - SR)/(MR - SR) \times 100$, where A is the mean of Vmax at a specific antibody concentration, SR is the mean of Vmax of the spontaneous release and MR is the mean of Vmax of the maximal release.

As additional readout the calcein retention of intact target cells was assessed by lysing the remaining target cells in borate buffer (5 mM sodium borate + 0.1 % Triton) and measuring the calcein fluorescence in a fluorescence plate reader. Mab205.10.1, Mab205.10.2, Mab205.10.3 showed ADCC [KPL-4] by 1µg/ml of specific Lysis of about 40-60%.

The afucosylated antibody (Mab205.10.1, Mab205.10.2, Mab205.10.3) were prepared by co-transfection with four plasmids, two for antibody expression, one for a fusion GnTIII polypeptide expression (a GnT-III expression vector), and one for mannosidase II expression (a Golgi mannosidase II expression vector) at a ratio of 4:4:1:1, respectively in HEK293 or CHO cells.

The full antibody heavy and light chain DNA sequences were subcloned into mammalian expression vectors (one for the light chain and one for the heavy chain)

under the control of the MPSV promoter and upstream of a synthetic polyA site, each vector carrying an EBV OriP sequence. Antibodies were produced by co-transfecting HEK293-EBNA cells or CHO cells with the antibody heavy and light chain expression vectors using a calcium phosphate-transfection approach.

5 Exponentially growing HEK293-EBNA cells were transfected by the calcium phosphate method. For the production of the glycoengineered antibody, the cells were co-transfected with four plasmids, two for antibody expression, one for a fusion GnTIII polypeptide expression (a GnT-III expression vector), and one for mannosidase II expression (a Golgi mannosidase II expression vector) at a ratio of
10 4:4:1:1, respectively. Cells were grown as adherent monolayer cultures in T flasks using DMEM culture medium supplemented with 10% FCS, and were transfected when they were between 50 and 80% confluent. For the transfection of a T150 flask, 15 million cells were seeded 24 hours before transfection in 25 ml DMEM culture medium supplemented with FCS (at 10% V/V final), and cells were placed
15 at 37°C in an incubator with a 5% CO₂ atmosphere overnight. For every antibody to be produced, a solution of DNA, CaCl₂ and water was prepared by mixing 188 µg total plasmid vector DNA (four plasmids, two for antibody expression (one light chain and one heavy chain), one for a fusion GnTIII polypeptide expression (a GnT-III expression vector), and one for mannosidase II expression (a Golgi
20 mannosidase II expression vector) at a ratio of 4:4:1:1, respectively), water to a final volume of 938 µl and 938 µl of a 1M CaCl₂ solution. To this solution, 1876 µl of a 50 mM HEPES, 280 mM NaCl, 1.5 mM Na₂HPO₄ solution at pH 7.05 were added, mixed immediately for 10 sec and left to stand at room temperature for 20 sec. The suspension was diluted with 46 ml of DMEM
25 supplemented with 2% FCS, and divided into two T150 flasks in place of the existing medium.

The cells were incubated at 37°C, 5% CO₂ for about 17 to 20 hours, then medium was replaced with 25 ml DMEM, 10% FCS. The conditioned culture medium was harvested 7 days post-transfection by centrifugation for 15 min at 210 x g, the
30 solution was sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % w/v was added, and kept at 4°C.

The secreted afucosylated antibodies were purified and the oligosaccharides attached to the Fc region of the antibodies were analysed e.g. by MALDI/TOF-MS (as described in e.g. WO 2008/077546). For this analysis oligosaccharides were
35 enzymatically released from the antibodies by PNGaseF digestion, with the antibodies being either immobilized on a PVDF membrane or in solution. The

resulting digest solution containing the released oligosaccharides either prepared directly for MALDI/TOF-MS analysis or was further digested with EndoH glycosidase prior to sample preparation for MALDI/TOF-MS analysis. The analyzed amount of fucose within the sugar chain at Asn297 was between 50-20%.

5 **Example 5**

HER2/HER3 and ER (estrogen receptor) crosstalk (cross-signaling)

HER2 and ER (estrogen receptor) crosstalk

5x10⁵ HEK 293 cells in DMEM 10% FCS per 6 well were seeded. 2µg of each cDNA was transfected with a standard Fugene protocol. Control vector, HER2 and ERα expressing cells were generated. After 48h cells were lysed by a Triton X100 lyses buffer containing Aprotinin 10µl/mL, PMSF 10µl/mL and Orthovanadate 2µl/ml. 20µg of total cell lysate was separated on a SDS PAGE and blotted against pERα (Cell Signaling mAb #2511, ERα (Cell Signaling mAb #8644) and HER2 (Calbiochem Ab 3) as indicated. All proteins are expressed at comparable levels. Only in cells expressing ERα and HER2 at the same time, ERα is phosphorylated on serine 118. Results are shown in Figure 2.

HER2 and ER complex

5x10⁵ HEK 293 cells in DMEM 10% FCS per 6 well were seeded. 2µg of each cDNA was transfected with a standard Fugene protocol. Control vector, HER2 and ERα expressing cells were generated. After 48h cells were lysed by a Triton X100 lyses buffer containing Aprotinin 10µl/mL, PMSF 10µl/mL and Orthovanadat 2µl/ml. Cells were immunoprecipitated with and anti HER2 antibody (trastuzumab) and blotted against ERα (Cell signaling mAb # 8644) or vice versa, immunoprecipitated with an anti ERα antibody (Cell signaling mAb # 8644) and blotted against HER2 (Calbiochem Ab 3). HER2 and ERα can form a complex and in this complex ERα is phosphorylated. Results are shown in Figure 3A,3B and 3C.

HER3 and ER (estrogen receptor) crosstalk

5x10⁵ HEK 293 cells in DMEM 10% FCS per 6 well were seeded. 2µg of each cDNA was transfected with a standard Fugene protocol. Control vector, HER3 and ERα expressing cells were generated. Cells were starved overnight in DEMEM 0, 5% FCS. After 48h cells were stimulated with HRG (black bars) for 10 min and

- 75 -

lysed by a Triton X100 lyses buffer containing Aprotinin 10µl/mL, PMSF 10µl/mL and Orthovanadate 2µl/ml. Cells were immunoprecipitated with anti HER3 antibody (Ab) and blotted against ERα antibody (Cell signaling mAb # 8644) or vice versa, immunoprecipitated with an anti ERα antibody (Cell signaling mAb # 8644) and blotted against HER3 (Ab208 Roche). HER3 and ERα can form a complex in the presence and absence of HRG, but ER is strongly phosphorylated only in the presence of HRG. Results are shown in Figure 4A,4B and 4C.

In summary: We could demonstrate this ER cross talk for HER2 and HER3, however no crosstalk could be detected for HER1 (data not shown). Several complementary findings support a model of HER2/HER3 and ER cross talk

- HER3 pathway is activated under long lasting tamoxifen therapy.
- HER3 and HER2 can form a complex with the estrogen receptor.
- HRG activated HER2/HER3 heterodimers mediate phosphorylation of estrogen receptor.
- HRG is an ER regulated gene.

ER signaling in breast cancer induces HRG expression which in turn drives mitogenic signaling via HER2/HER3 heterodimers. HER2 can activate estrogen receptor

Example 6

In vivo antitumor efficacy of anti-HER3 therapy in combination with pertuzumab

The in vivo antitumor efficacy of the antibody Mab205.10.2 in combination with pertuzumab was detected in a fragment based HER2 low, ER+, HER3 + breast cancer patient-derived xenograft model (PDX) transplanted on nude mice. As example data is shown for the HBCx-19 PDX xenograft model.

Test agents

Mab205.10.2 and pertuzumab were provided as stock solution from Roche, Penzberg, Germany. Antibody buffer included histidine. Antibody solution was diluted appropriately in buffer from stock prior injections.

Tumor fragments

HBCx-19 breast cancer tumor fragments were originally obtained from a metastasis of a lobular carcinoma. Tumors of the same passage were transplanted subcutaneously onto 6-24 donor mice. When these tumors reached 1000 to 2000 mm³, donor mice were sacrificed and tumors excised, dissected and cut into fragments measuring of approximately 20 mm³. Thereafter fragments were grafted subcutaneous.

Animals

Female athymic nude mice, 6- to 9-week-old at the beginning of the experimental phase, were obtained from *Harlan Laboratories* (France). Animals were maintained in specific pathogen-free animal housing at the Center for Exploration and Experimental Functional Research (CERFE, Evry, France) animal facility. Animals were delivered to the laboratory 7 days before the experiments during which time they were acclimatized to laboratory conditions. Mice were housed inside individually ventilated cages (IVC) under a light-dark cycle (14-hour circadian cycle of artificial light) and controlled room temperature and humidity.

Monitoring

Animals were controlled daily for clinical symptoms and detection of adverse effects. For monitoring throughout the experiment body weight of animals was documented.

Treatment of animals

Animal treatment started at randomisation (D26) when median tumor size was about 100–150mm³. Antibody Mab205.10.2 was administered as single agent and in combination with pertuzumab at 10 mg/kg i.p. q7d once weekly until D61. Pertuzumab was administered as single agent and in combination once weekly at 15 mg/kg with a 2-fold loading dose first. The corresponding vehicle was administered on the same days.

Antibody efficacy

HBCx-19 breast cancer xenograft model

HBCx-19 breast cancer xenograft bearing mice were treated with antibody Mab205.10.2 as single agent and in combination with pertuzumab from study D26 to D61. As a result, treatment with Mab205.10.2 antibody as single agent showed strong significant anti-tumor efficacy with tumors stasis until D61 and a Relative Tumor Volume (RTV) of 100%. Monotherapy with pertuzumab translated into partial HBCx-19 tumor regression at 61%. Notably, superior efficacy was observed with Mab205.10.2 in combination with pertuzumab resulting in strongest tumor regression (RTV 10%).

Antibody treatment discontinued at D61. In the follow-up tumor regrowth of HBCx-19 xenografts was observed in both monotherapy groups with Mab205.10.2 or pertuzumab (RTV 728% and 131%, respectively), however, in contrast to this combination of Mab205.10.2 with pertuzumab maintained long lasting tumor remission (RTV 10%). Results are shown in Figure 5.

Example 7

In vivo antitumor efficacy of anti-HER3 therapy in combination with pertuzumab and anti-hormone therapy

The in vivo antitumor efficacy of the antibody Mab205.10.2 in combination with pertuzumab and selective estrogen receptor degrader (SERD) fulvestrant was detected in a fragment based HER2 low, ER+, HER3+ breast cancer patient-derived xenograft model (PDX) transplanted on nude mice. As example data is shown for the HBCx-19 PDX xenograft model.

Test agents

Mab205.10.2 and pertuzumab were provided as stock solution from Roche, Penzberg, Germany. Antibody buffer included histidine. Antibody solution was diluted appropriately in buffer from stock prior injections. Fulvestrant (Faslodex, Astra Zeneca) was purchased commercially.

Tumor fragments

HBCx-19 breast cancer tumor fragments were originally obtained from a metastasis of a lobular carcinoma. Tumors of the same passage were transplanted

subcutaneously onto 6-24 donor mice. When these tumors reached 1000 to 2000 mm³, donor mice were sacrificed and tumors excised, dissected and cut into fragments measuring of approximately 20 mm³. Thereafter fragments were grafted subcutaneous.

5 **Animals**

Female athymic nude mice, 6- to 9-week-old at the beginning of the experimental phase, were obtained from *Harlan Laboratories* (France). Animals were maintained in specific pathogen-free animal housing at the Center for Exploration and Experimental Functional Research (CERFE, Evry, France) animal facility.

10 Animals were delivered to the laboratory 7 days before the experiments during which time they were acclimatized to laboratory conditions. Mice were housed inside individually ventilated cages (IVC) under a light-dark cycle (14-hour circadian cycle of artificial light) and controlled room temperature and humidity.

Monitoring

15 Animals were controlled daily for clinical symptoms and detection of adverse effects. For monitoring throughout the experiment body weight of animals was documented.

Treatment of animals

20 Animal treatment started at randomisation (D22) when median tumor size was about 100–150mm³. Antibody Mab205.10.2 was administered at 3 mg/kg as single agent once weekly (6x) as single agent and in dual combination with pertuzumab (3 mg/kg i.p. q7dx6) or in triple combination with fulvestrant (50 mg/kg, im, q7dx6) until D57. Pertuzumab was administered as single agent and in combination once weekly at 3 mg/kg and fulvestrant given im at 50 mg/kg once weekly. The

25 corresponding vehicle was administered on the same days.

Antibody efficacy

HBCx-19 breast cancer xenograft model

HBCx-19 HER2 low, ER+, HER3+ breast cancer xenograft bearing mice were treated with antibody Mab205.10.2 as single agent and in combination with

30 pertuzumab and anti-estrogen fulvestrant from study D22 to D57. The triple combination (Mab205.10.2 + pertuzumab + fulvestrant) was the most efficacious

combination with tumor control of HBCx-19 BC xenografts (RTV 118%). The monotherapies and dual combinations were less efficacious and HBCx-19 tumors progressed. Results are shown in Figure 6.

Example 8

5 In vivo antitumor efficacy of anti-HER3 therapy (glycoengineered Mab205.10.2) in combination with pertuzumab and chemotherapy (paclitaxel) (clinical trial data)

Glycoengineered Mab205.10.2 was investigated in combination with pertuzumab and paclitaxel in patients with MBC expressing HER3 and HER2 protein.

10 Anti-HER3 antibody Mab205.10.2 was administered at a dose of 500 mg on a q3w schedule (i.e., on day 1 of 3-weekly cycles). Pertuzumab was administered at a loading dose of 840 mg during cycle 1 followed by 420 mg for the remaining cycles in a q3w schedule. Paclitaxel was be administered at 80 mg/m² in a qw schedule. In another setting at a dose of Mab205.10.2 of 1000 mg q3w diarrhea as
15 side effect could be detected. By a reduced dose of Mab205.10.2 of 500 mg q3w in comparison with 1000 mg q3w schedule diarrhea e.g. could be significantly reduced. Results in terms of RECIST criterias (SD = Stable Disease, PR = Partial Response, CR = Complete Response, PD = Progressive Disease; ORR =Overall Response Rate, DCR Disease Control rate, are shown in Figures 7 and 8.

20 Figure 7: In vivo antitumor efficacy (reduction of lesions) anti-HER3-antibody Mab205.10.2 in combination with pertuzumab **in pretreated** (second (2L) to fourth line (4L) **and first line** (1L) HER2 low, ER+, HER3 + metastatic breast cancer patients. All patients are HER2 low, ER+, HER3 + except patients 1071, 1072 and 1375 who are estrogen receptor negative (ER-). Results in terms of
25 RECIST criterias (SD = Stable Disease, PR = Partial Response, CR = Complete Response, PD = Progressive Disease; ORR =Overall Response Rate, DCR Disease Control rate. The best overall RECIST responses and the line of treatment are indicated.

Figure 8: In vivo antitumor efficacy (reduction of lesions) anti-HER3-antibody
30 Mab205.10.2 in combination with pertuzumab **in only first line (1L)** HER2 low, ER+, HER3 + metastatic breast cancer patients. All patients are HER2 low, ER+, HER3 + Results in terms of RECIST criterias (SD = Stable Disease, PR = Partial Response, CR = Complete Response, PD = Progressive Disease; ORR =Overall

- 80 -

Response Rate, DCR Disease Control rate. The best overall RECIST responses and the line of treatment are indicated.

5 In another setting Mab205.10.2 was administered at a dose of 500 mg on a q3w schedule (i.e., on day 1 of 3-weekly cycles). Pertuzumab was administered at a dose of 420 mg in a q3w schedule. Paclitaxel was be administered at 80 mg/m² in a qw schedule (data not shown). By a reduced dose of Mab205.10.2 of 500 mg q3w in comparison with 1000 mg q3w schedule side effects like diarrhea could be significantly reduced.

Patent Claims

1. An antibody which binds to human HER3 for use in the treatment of breast cancer in combination with an antibody binding to human HER2 and inhibiting dimerization of HER2, wherein the breast cancer is HER2 low,
5 HER3 positive and HR (hormone receptor) positive.
2. The antibody according to claim 1, wherein the breast cancer is HER2 low HER3, and positive ER (estrogen receptor) positive .
3. The antibody according to any one of claims 1 to 2, wherein additionally either
10 A) an anti-hormonal agent is co-administered, wherein the anti-hormonal agent is selected from the group of:
 - a) a selective estrogen receptor degrader (SERD), or
 - b) a selective estrogen receptor modulators (SERM); and such as tamoxifen, raloxifene, toremifene or lasofoxifene, or
 - 15 c) an aromatase inhibitor;
or
B) a chemotherapeutic agent is co-administered, wherein the chemotherapeutic agent is selected from the group of:
 - a) a taxane or taxane derivative
 - 20 b) cyclophosphamide,
 - c) cisplatin or carboplatin
 - d) mitomycin C,
 - e) methotrexate,
 - f) 5-fluorouracil or capecitabine,
 - 25 g) doxorubicin or its liposomal formulation, or daunorubicin or its liposomal formulation,

- 82 -

- h) gemcitabine,
 - i) mitoxantrone,
 - j) vinorelbine,
 - k) eribulin, and
 - 5 l) ixabepilone.
4. The antibody according to any one of claims 1 to 2, wherein additionally an anti-hormonal agent is co-administered, and wherein the anti-hormonal agent is selected from the group of:
- a) a selective estrogen receptor degrader (SERD),
 - 10 b) a selective estrogen receptor modulator (SERM), and
 - c) an aromatase inhibitor.
5. The antibody according to claim 4, wherein the anti-hormonal agent is selected from the group of:
- a) fulvestrant,
 - 15 b) tamoxifen, raloxifene, toremifene or lasofoxifene, and
 - c) letrozole, anastrozole or exemestane.
6. The antibody according to claim 4, wherein the anti-hormonal agent is fulvestrant.
7. The antibody according to any one of claims 1 to 2, wherein additionally a chemotherapeutic agent is co-administered, and wherein the chemotherapeutic agent is selected from the group of:
- 20 a) a taxane or taxane derivative
 - b) cyclophosphamide,
 - c) cisplatin or carboplatin
 - 25 d) mitomycin C,

- 83 -

- e) methotrexate,
 - f) 5-fluorouracil or capecitabine,
 - g) doxorubicin or its liposomal formulation, or daunorubicin or its liposomal formulation,
 - 5 h) gemcitabine,
 - i) mitoxantrone,
 - j) vinorelbine,
 - k) eribulin, and
 - l) ixabepilone.
- 10 8. The antibody according claim 7, wherein the chemotherapeutic agent is a taxanes or taxane derivative selected from the group of paclitaxel, nab-paclitaxel and docetaxel.
9. The antibody according to any one of claims 1 to 8, wherein the combination is administered as a first-line treatment.
- 15 10. The antibody according to any one of claims 1 to 9, wherein the antibody which binds to human HER3 is characterized in comprising as heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.
- 20 11. The antibody according to any one of claims 1 to 9, wherein the antibody which binds to human HER3 is characterized in that
- the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:10.
- 25 12. The antibody according to any one of claims 1 to 11, wherein the antibody which binds to human HER3 is of human IgG1 subclass and is characterized in that the antibody is glycosylated with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65 % or lower.

- 84 -

13. The antibody according to any one of claims 1 to 12, wherein the antibody binding to human HER2 and inhibiting dimerization of HER2 is pertuzumab.
 14. The antibody according to any one of claims 1 to 13, wherein the antibody which binds to human HER3 is administered at a dose of 400-600 mg every 3 weeks and the antibody which binds to human HER2 is administered at a dose of 300-500 mg every 3 weeks.
- 5

Fig. 1A

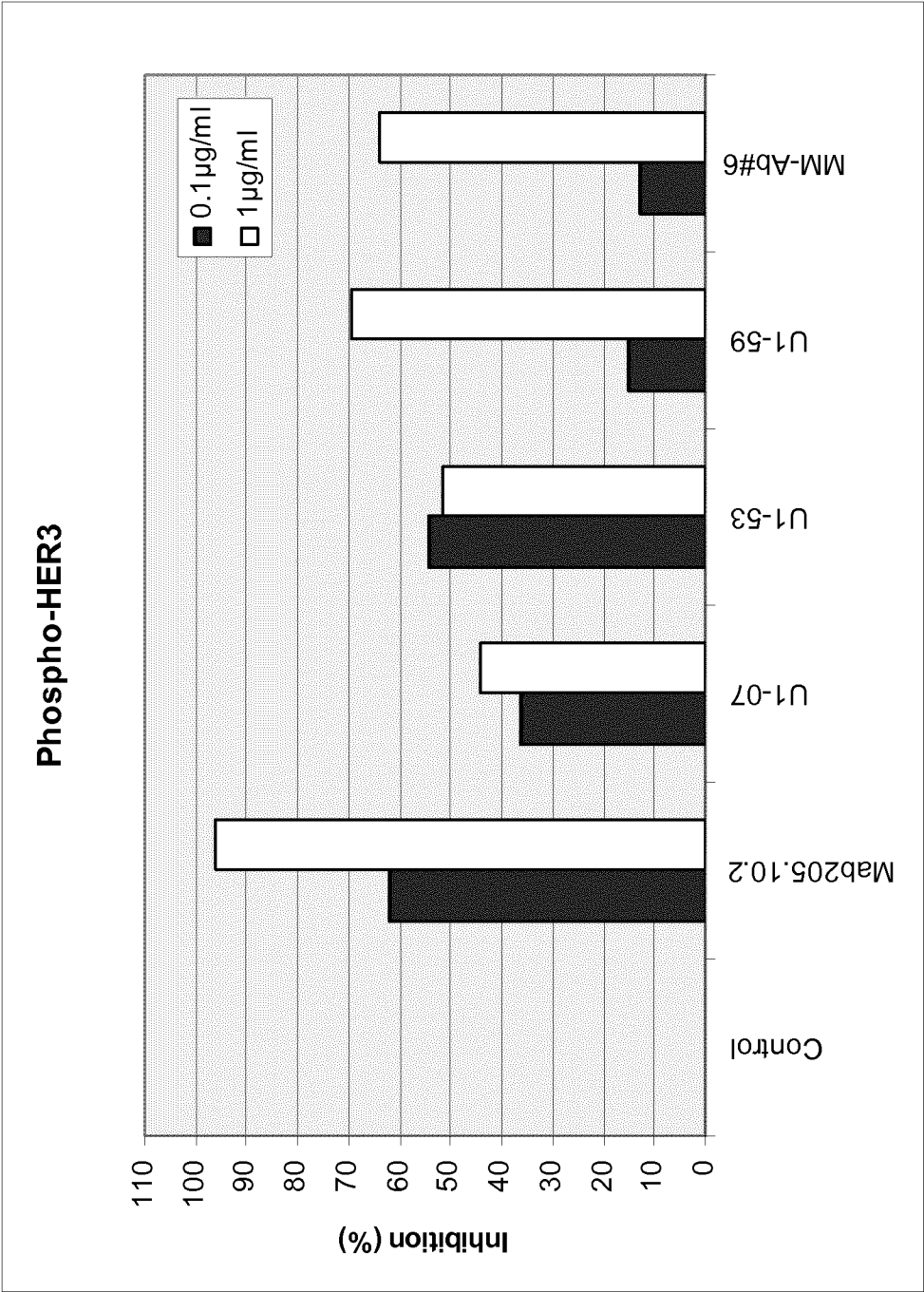


Fig. 1B

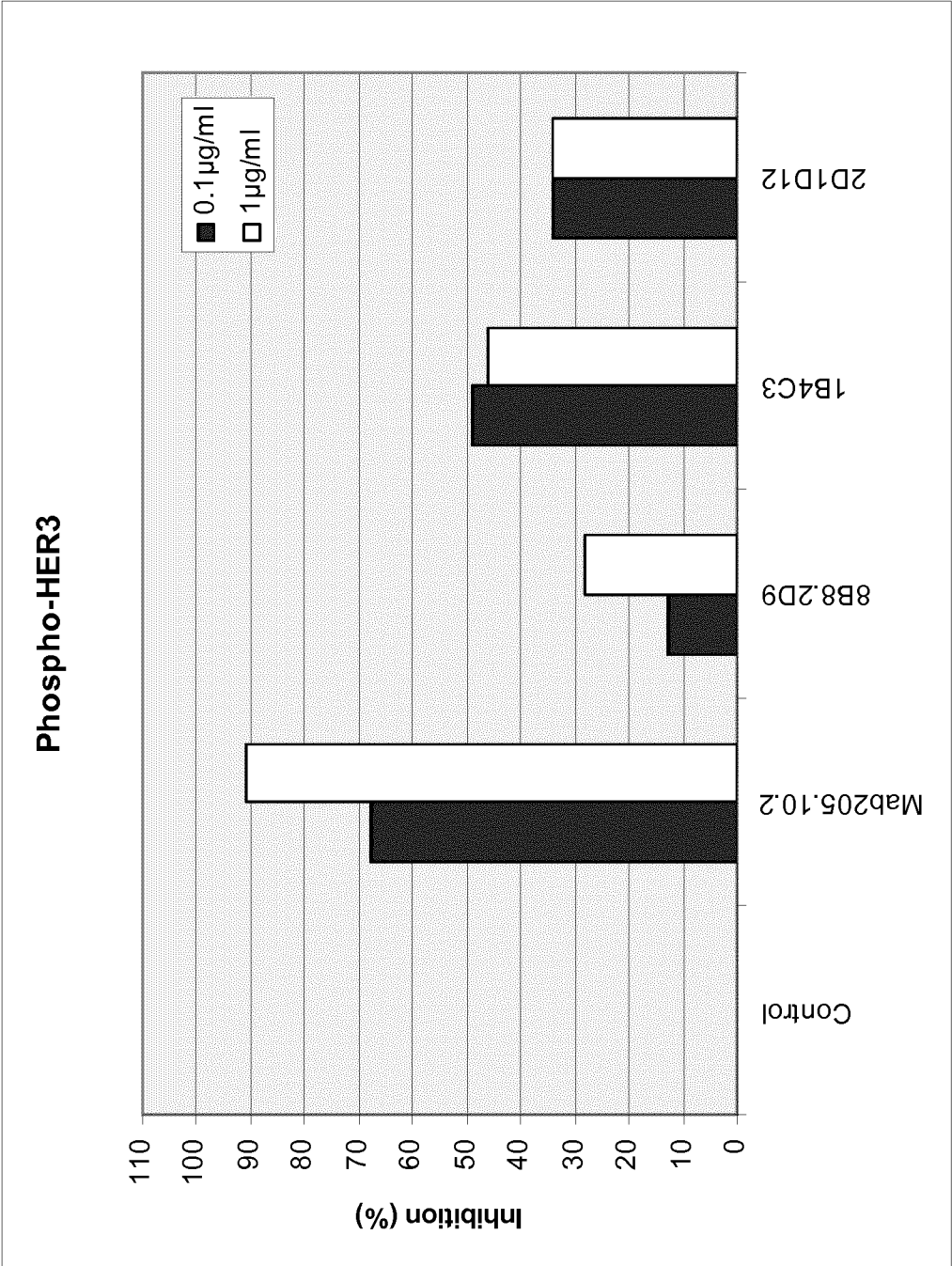


Fig. 1C

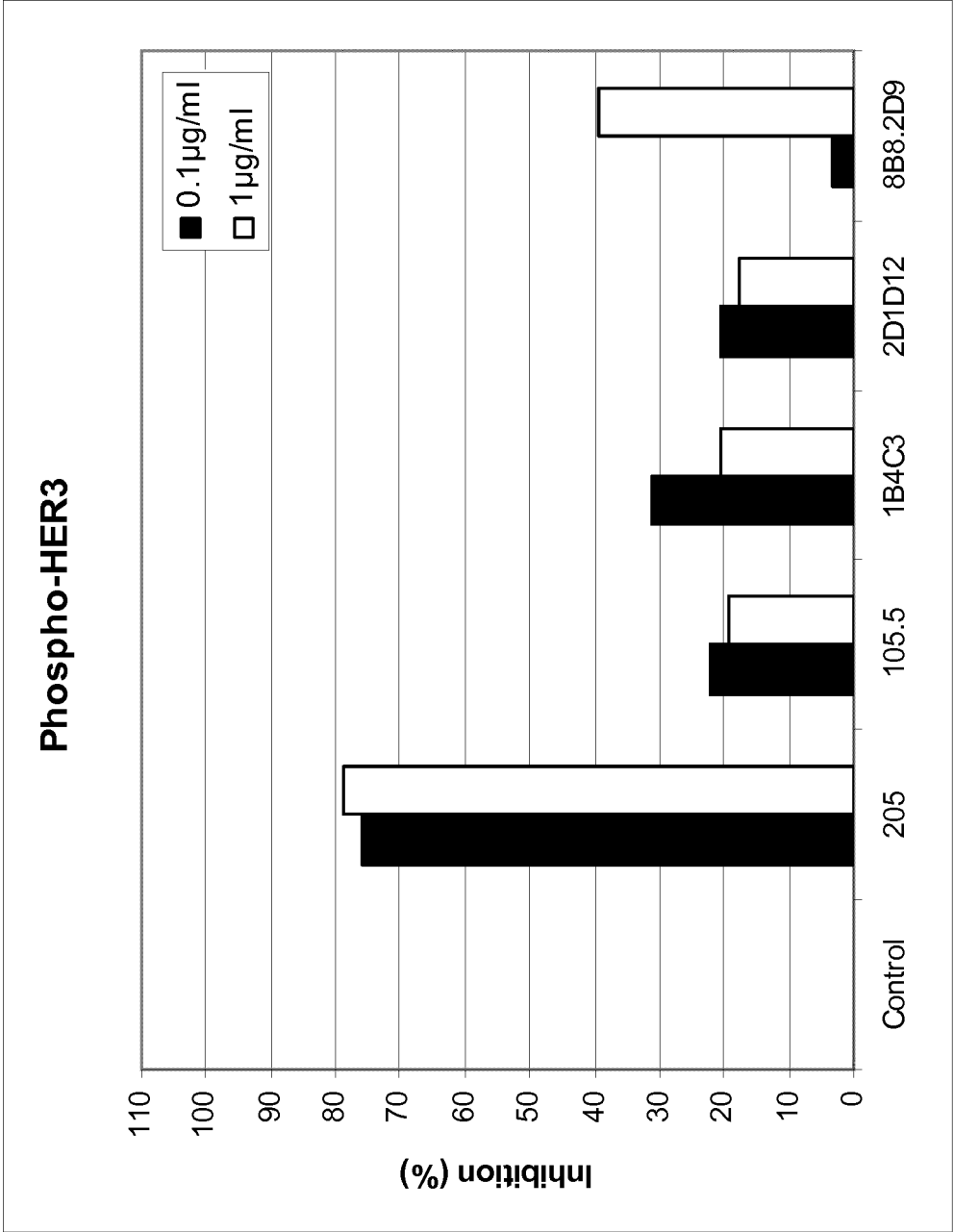


Fig. 2

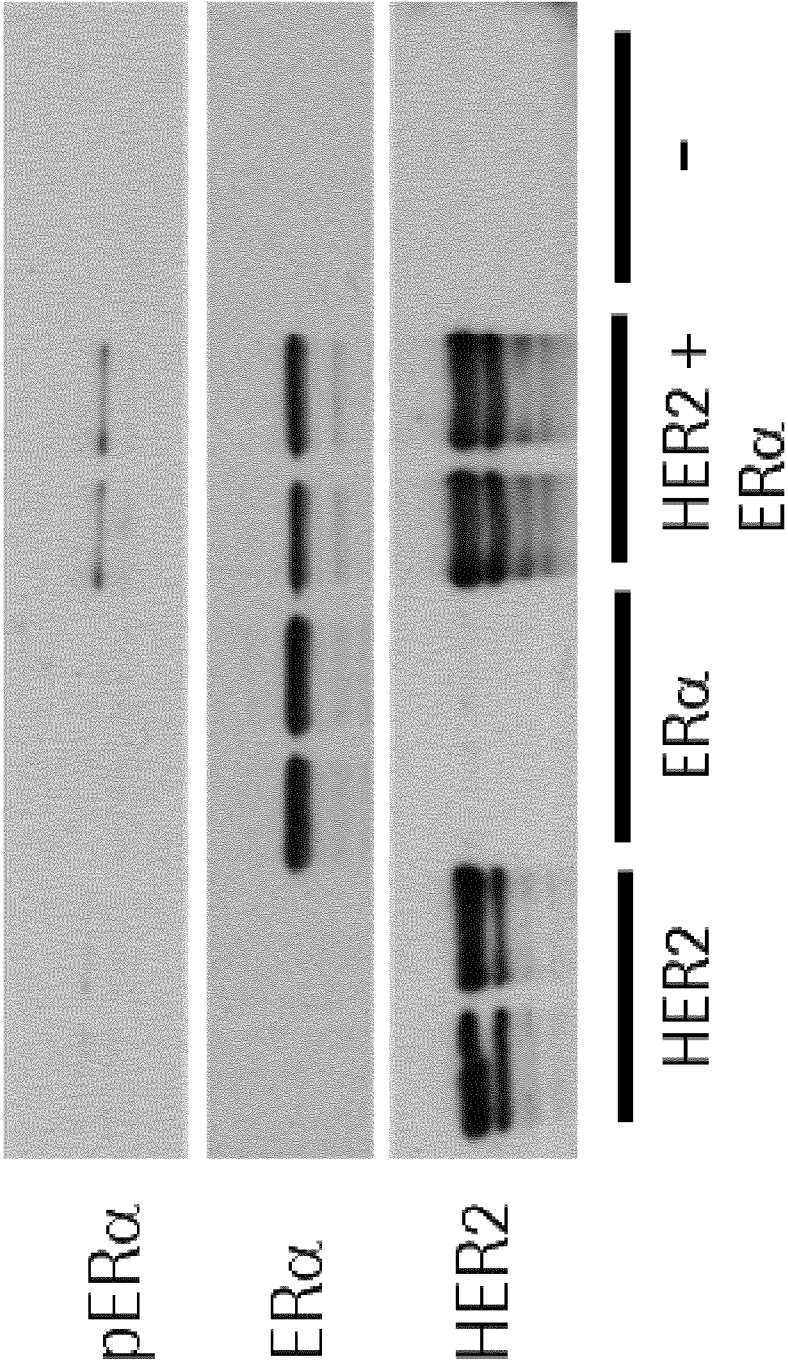


Fig. 3A

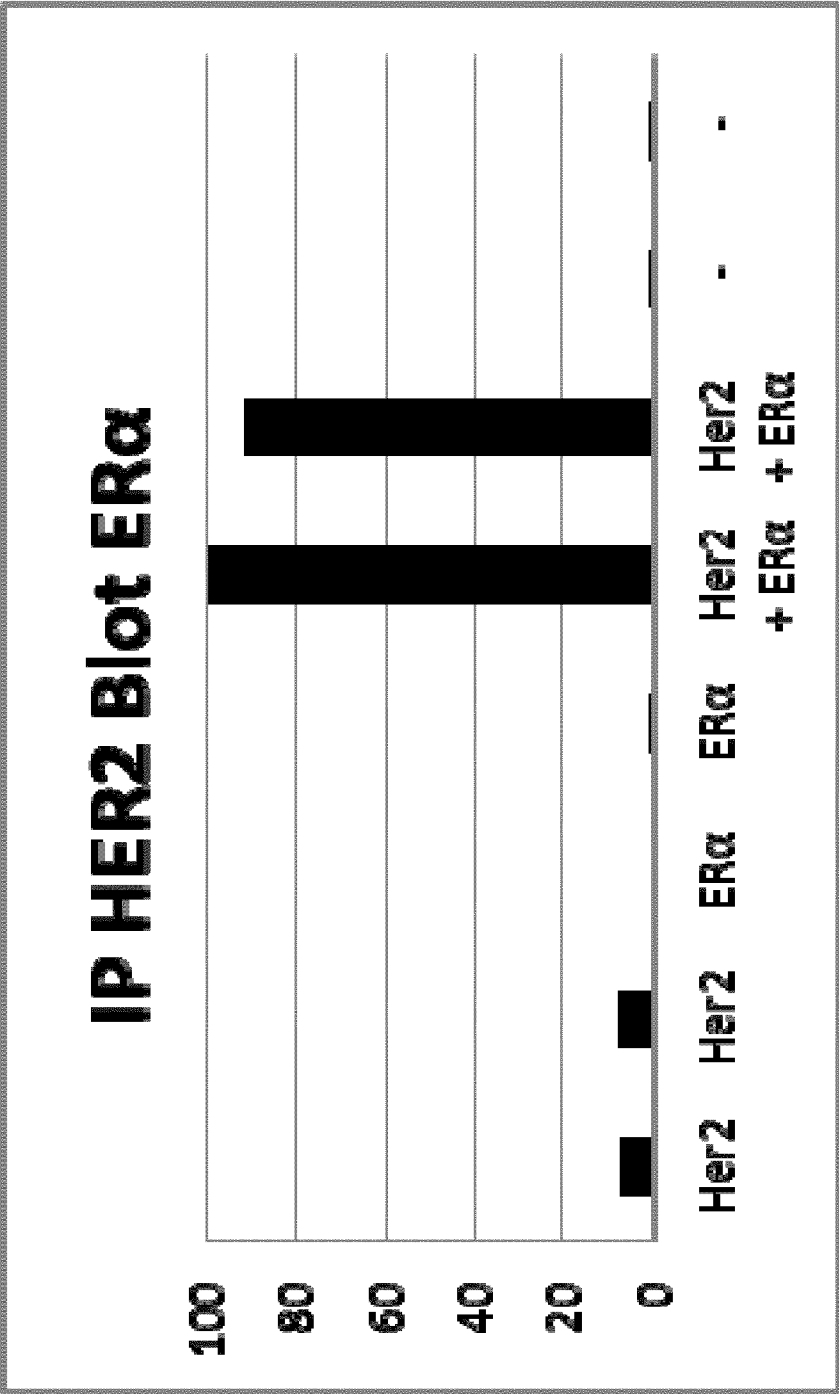


Fig. 3B

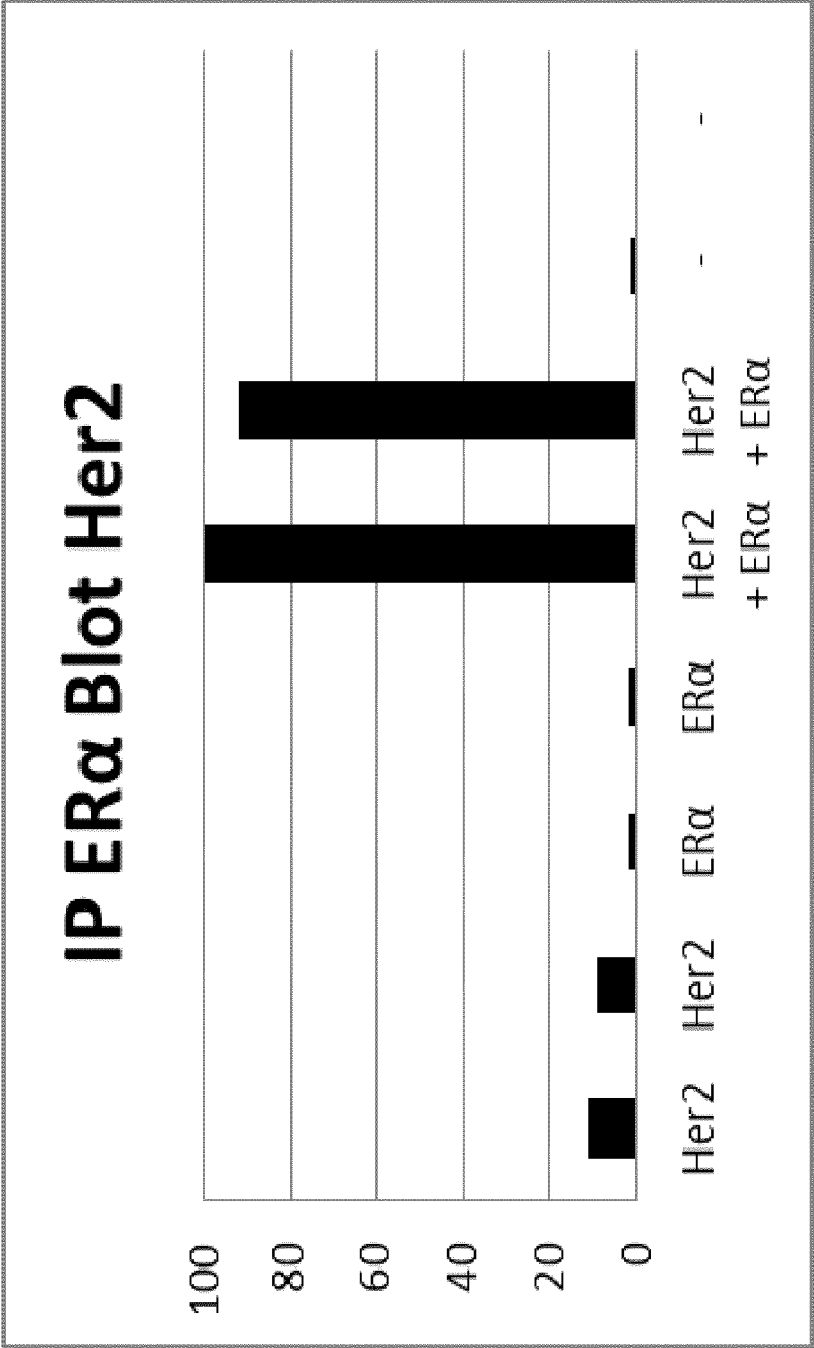


Fig. 3C

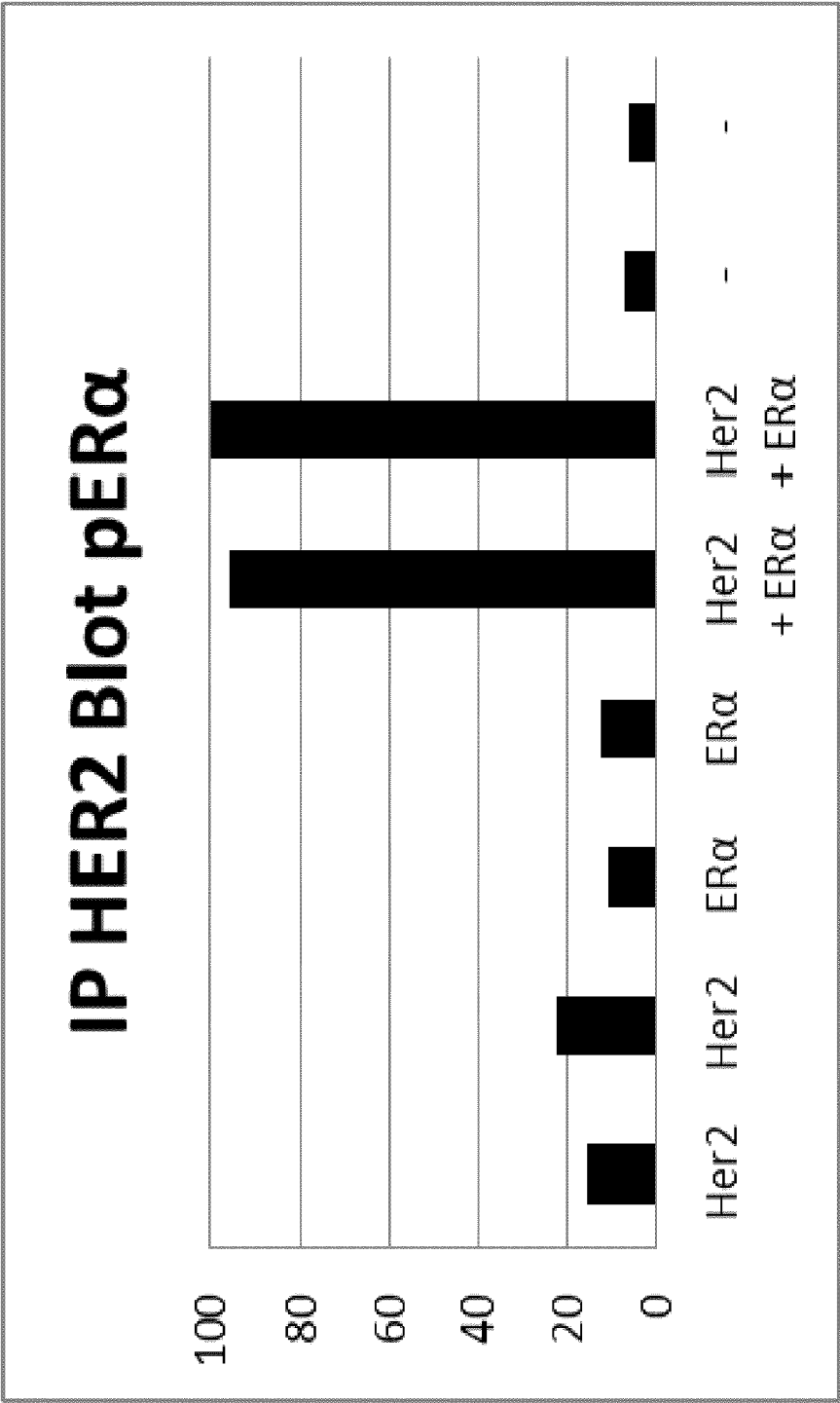


Fig. 4A

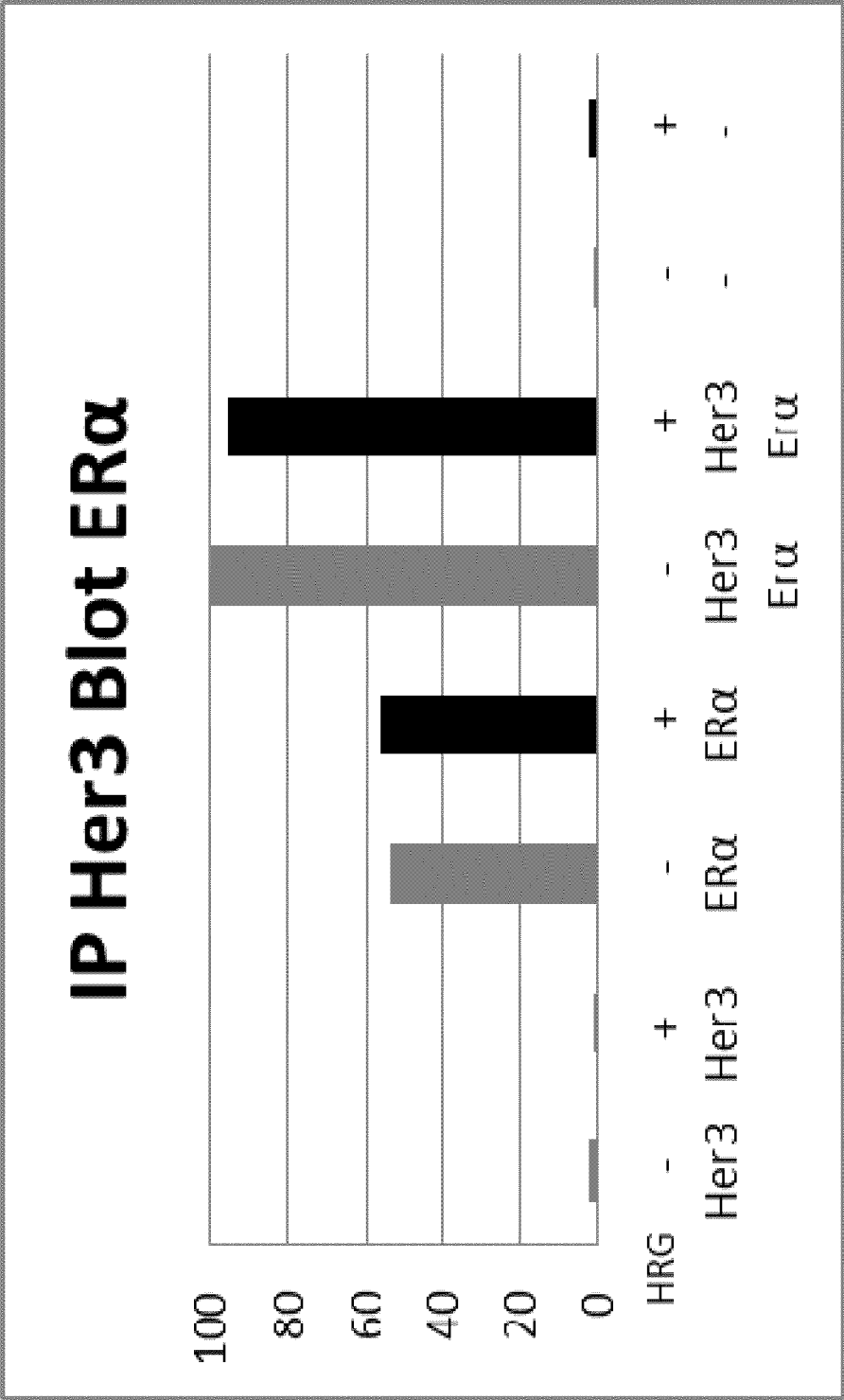


Fig. 4B

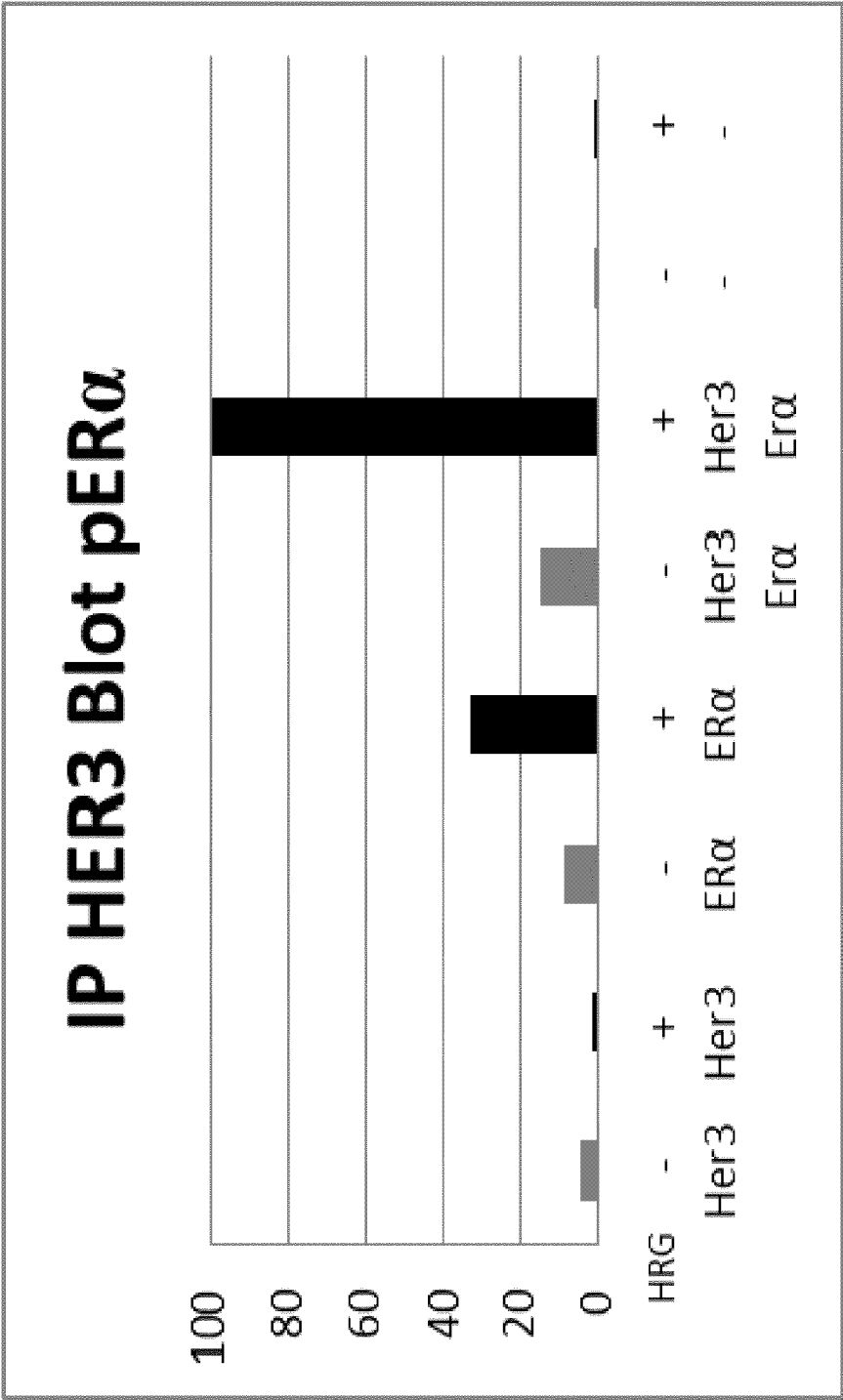


Fig. 4C

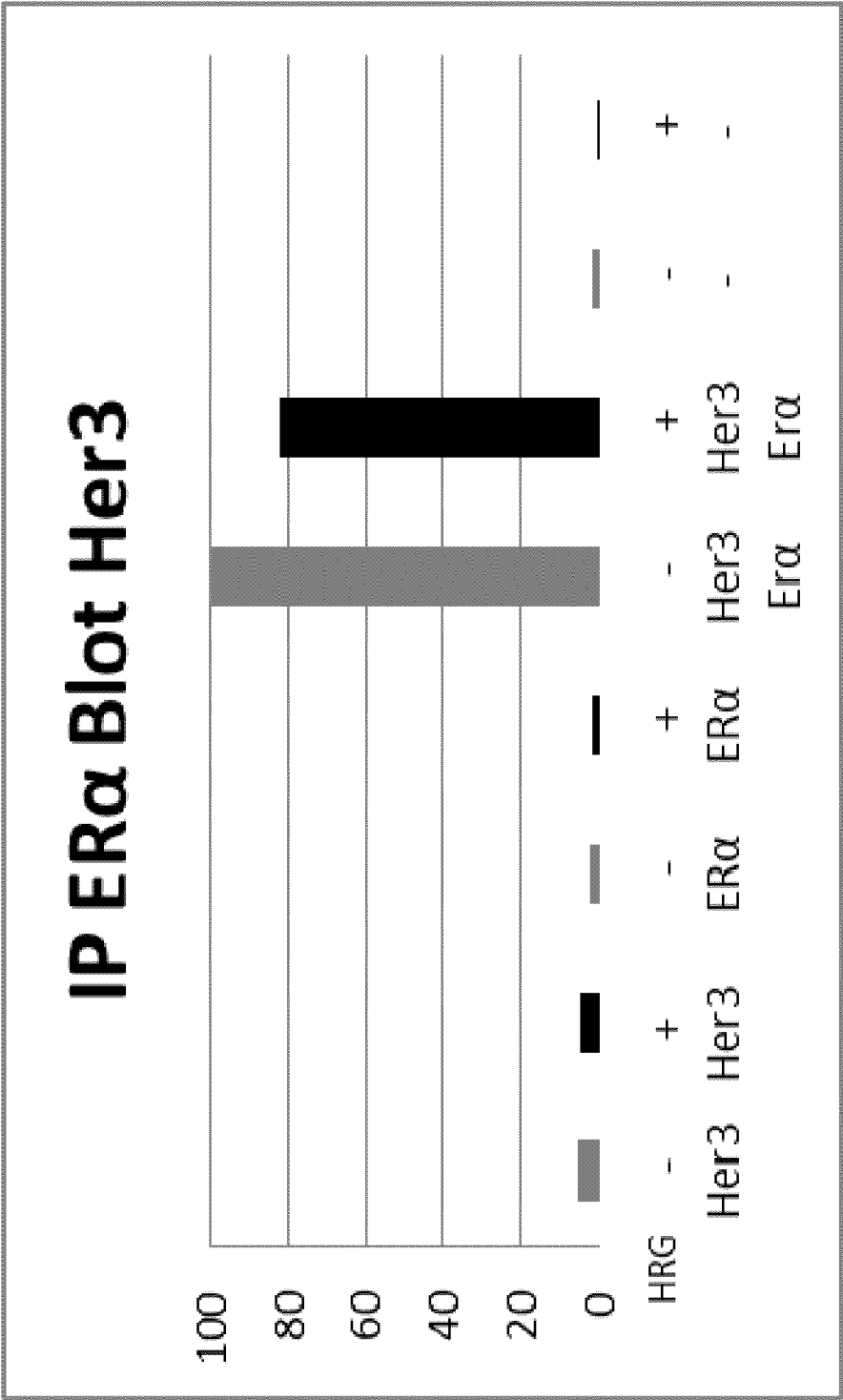


Fig. 5A

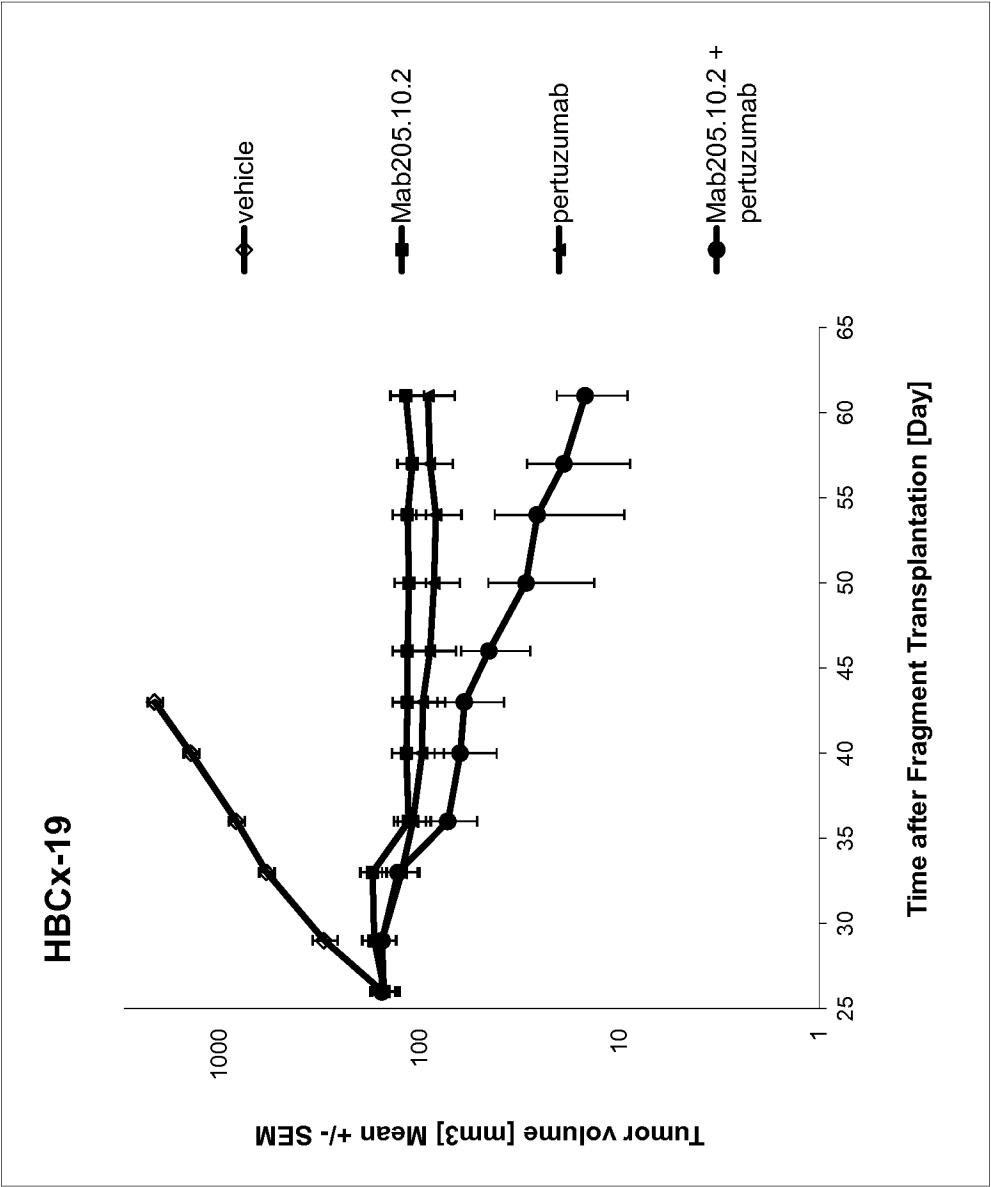


Fig. 5B

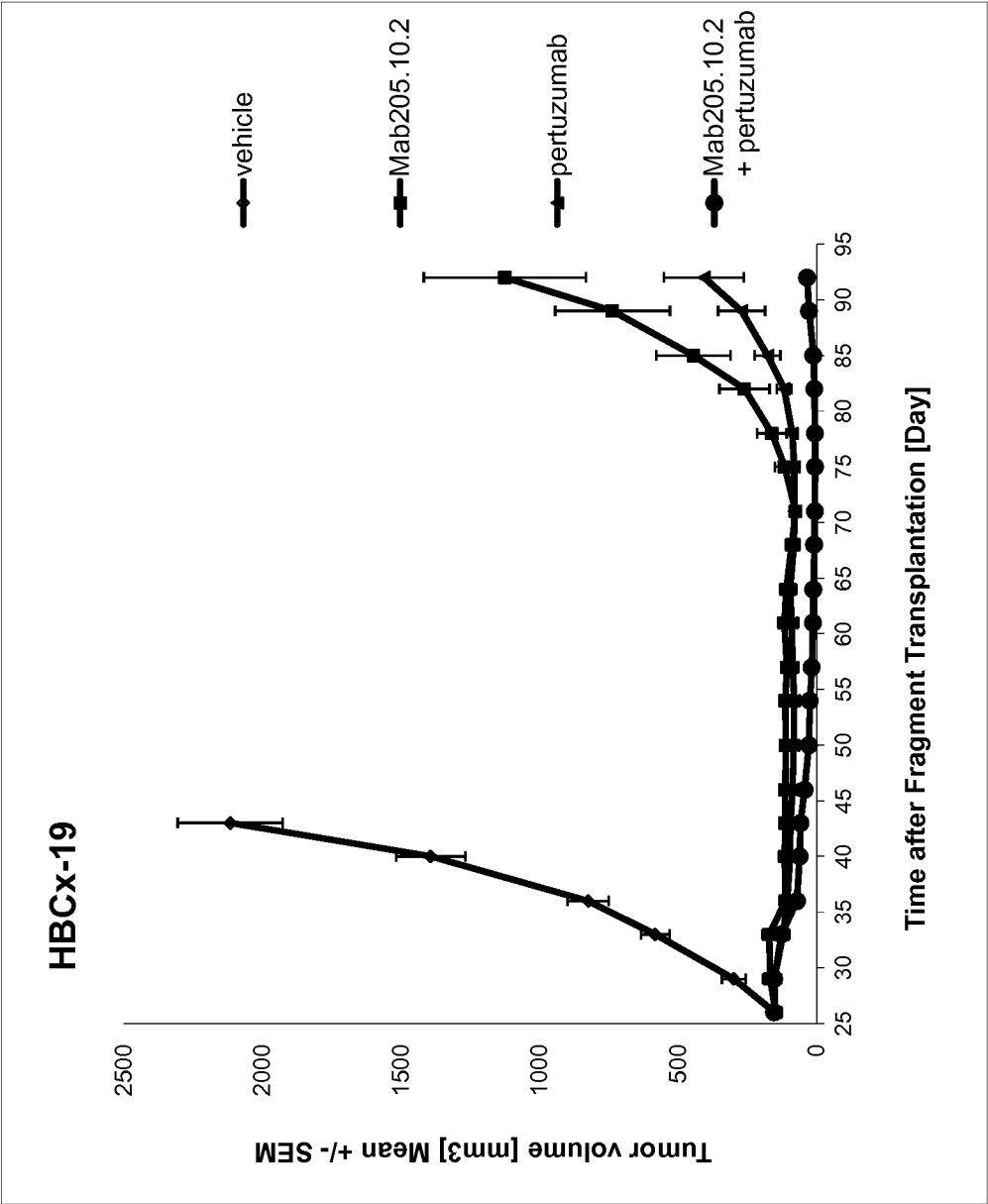


Fig. 5C

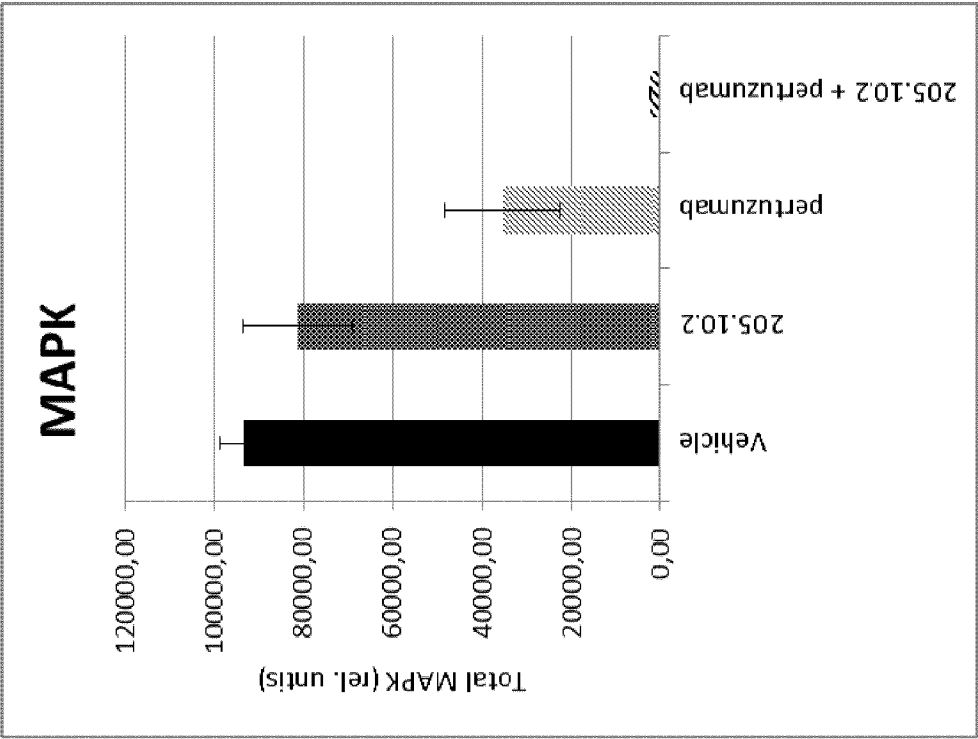


Fig. 5D

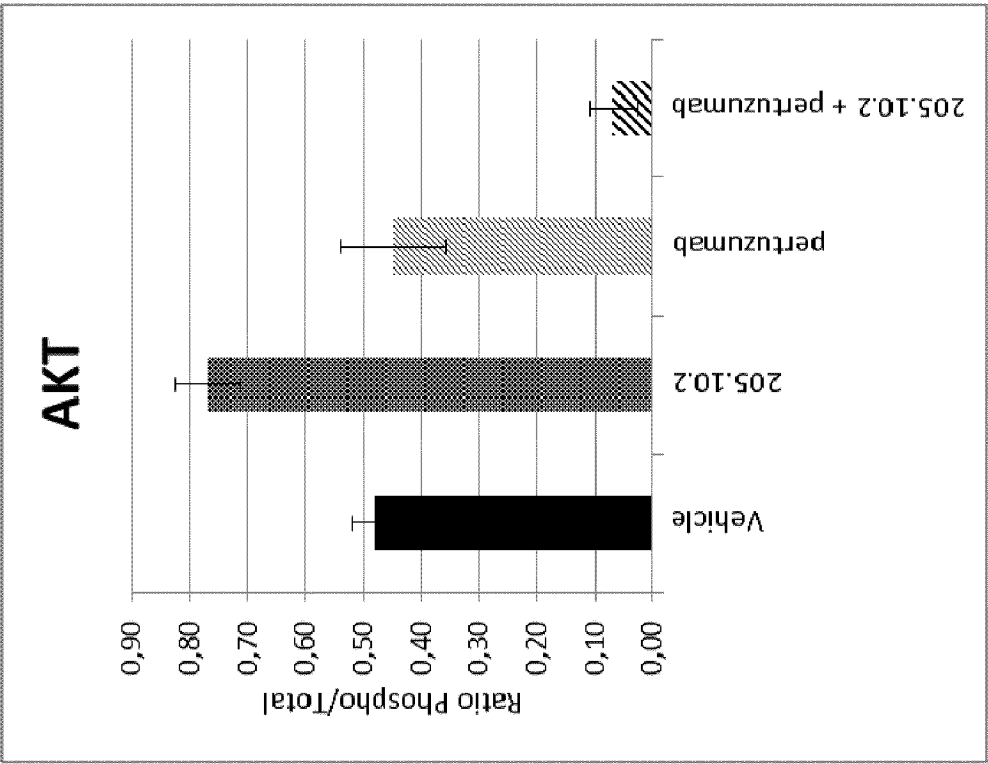


Fig. 6

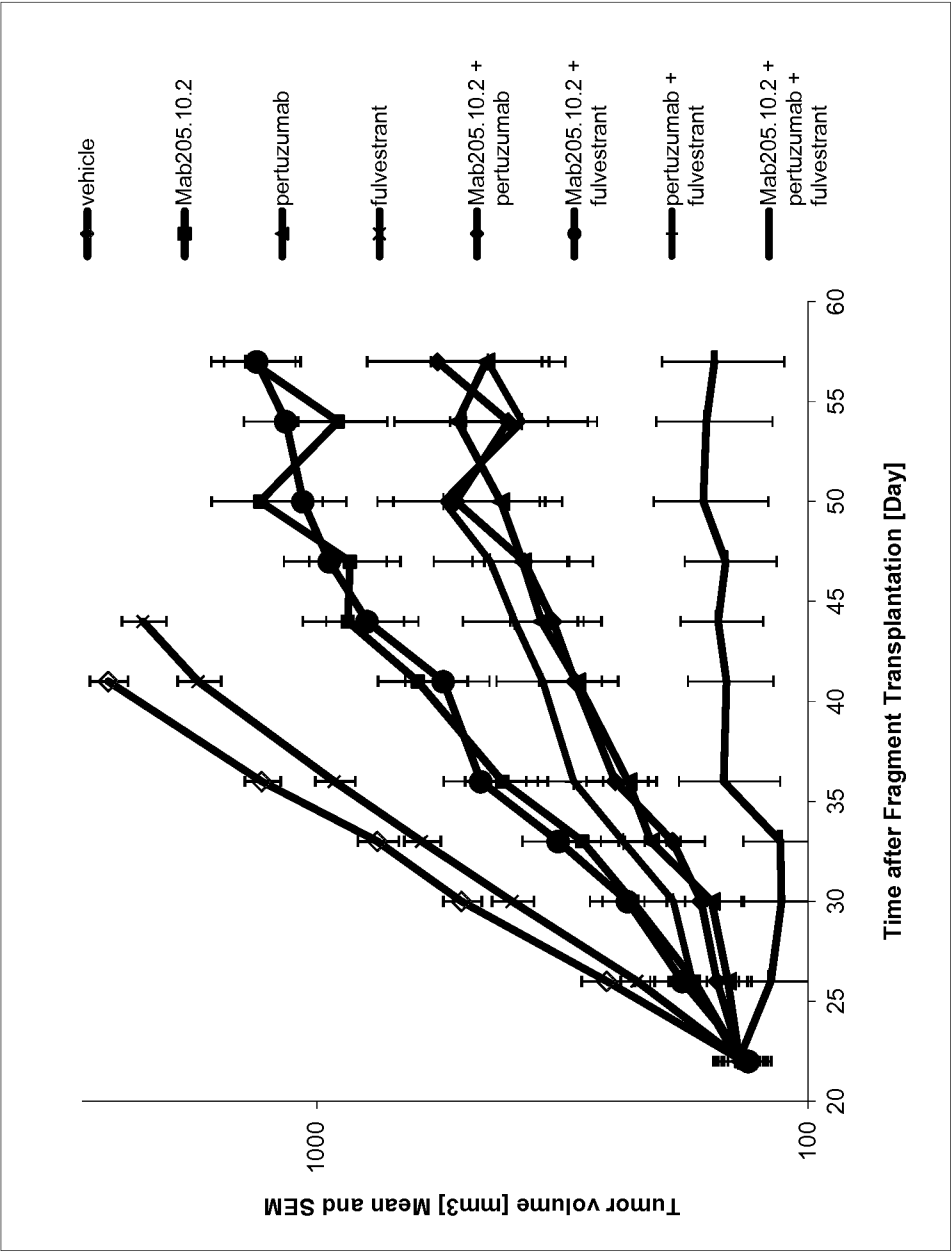


Fig. 7

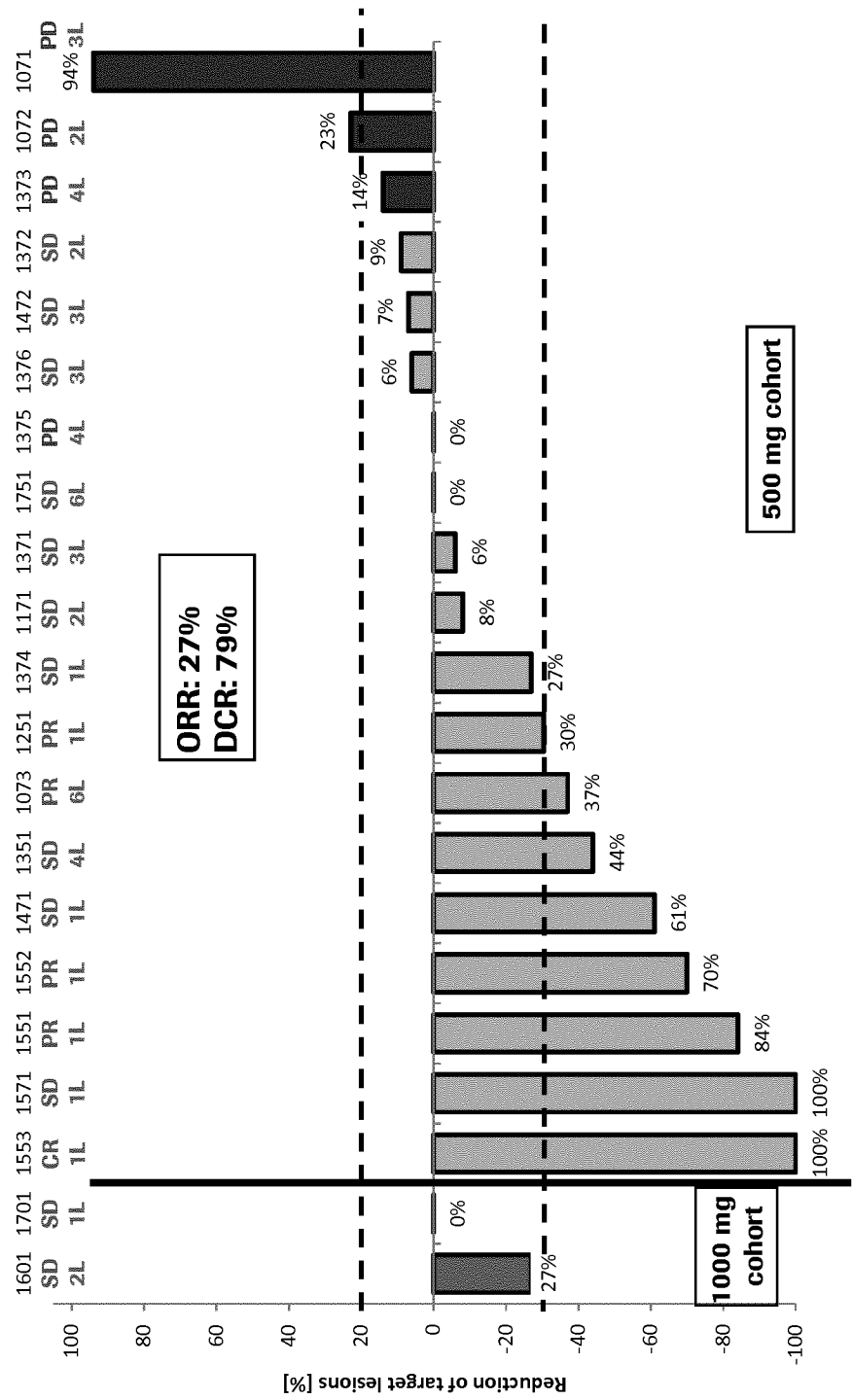
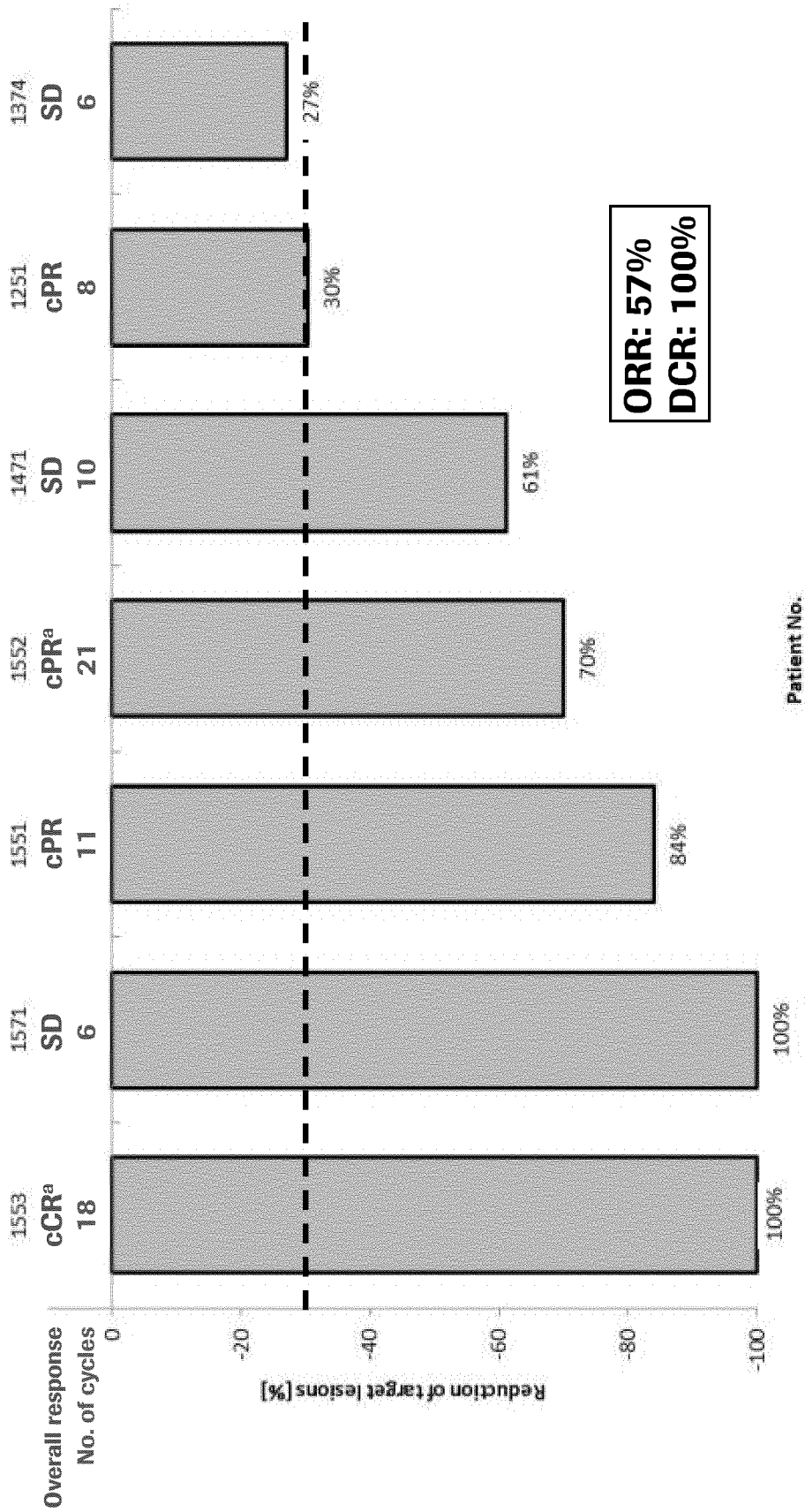


Fig. 8



Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/080890

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/395 A61K31/337 A61K31/565 C07K16/32 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/108484 A2 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 17 July 2014 (2014-07-17) page 35, line 13 - page 37, line 12; claims 1-16; examples 1,7,8 -----	1-14
X	WO 2014/036520 A1 (MERRIMACK PHARMACEUTICALS INC [US]) 6 March 2014 (2014-03-06) claims 1-29; examples 16-22 -----	1-14
X	WO 2011/060206 A2 (U3 PHARMA GMBH [DE]; AMGEN [US]; HETTMANN THORE [DE]; FREEMAN DANIEL J) 19 May 2011 (2011-05-19) examples 19,27,28 ----- -/--	1-14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

15 February 2017

Date of mailing of the international search report

23/02/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Siaterli, Maria

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/080890

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/182970 A1 (ZYMEWORKS INC [CA]; NG GORDON YIU KON [CA]) 13 November 2014 (2014-11-13) examples 24-29	1-14
X	----- CARRARO S ET AL: "Phase 1b/2 trial of the HER3 inhibitor patritumab (U3-1287) in combination with trastuzumab plus paclitaxel in newly-diagnosed patients with HER2+metastatic breast cancer (MBC)", CANCER RESEARCH, vol. 73, no. Suppl. 24, December 2013 (2013-12), pages P4-16, XP002756797, & 36TH ANNUAL SAN ANTONIO BREAST CANCER SYMPOSIUM; SAN ANTONIO, TX, USA; DECEMBER 10 -14, 2013 ISSN: 0008-5472 abstract	1-14
X	----- Zhang: "Abstract 655: Combination of MM-111, an ErbB2/ErbB3 bispecific antibody, with endocrine therapies as an effective strategy for treatment of ER+/HER2+ breast cancer", Cancer Research, 2 April 2011 (2011-04-02), pages 1-2, XP055266204, DOI: 10.1158/1538-7445.AM2011-655 Retrieved from the Internet: URL:http://cancerres.aacrjournals.org/content/71/8_Supplement/655.abstract?sid=afc6d588-67e3-486b-84d2-c41c76637228 [retrieved on 2016-04-18] the whole document	1-14
X	----- Anonymous: "A Study to Evaluate R05479599 in Combination With Pertuzumab (Perjeta) and Paclitaxel in Participants With Metastatic Breast Cancer Expressing Human Epidermal Growth Factor (HER) 3 & HER2 Protein", ClinicalTrials.gov archive, 1 December 2015 (2015-12-01), pages 1-6, XP055267046, Retrieved from the Internet: URL:https://clinicaltrials.gov/archive/NCT01918254/2015_12_01 [retrieved on 2016-04-20] the whole document -----	1-3,7-9, 12-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2016/080890

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014108484	A2	17-07-2014	AR 094403 A1 29-07-2015
			AU 2014204795 A1 30-04-2015
			CA 2887508 A1 17-07-2014
			CL 2015001049 A1 16-10-2015
			CN 104797602 A 22-07-2015
			CR 20150284 A 30-06-2015
			EA 201500744 A1 30-12-2015
			EP 2943508 A2 18-11-2015
			HK 1211950 A1 03-06-2016
			JP 2016504399 A 12-02-2016
			KR 20150093231 A 17-08-2015
			PH 12015501071 A1 03-08-2015
			SG 11201503802U A 28-08-2015
			TW 201431559 A 16-08-2014
			US 2014227255 A1 14-08-2014
			US 2016159912 A1 09-06-2016
			WO 2014108484 A2 17-07-2014
WO 2014036520	A1	06-03-2014	US 2014079703 A1 20-03-2014
			US 2015202287 A1 23-07-2015
			US 2016326262 A1 10-11-2016
			WO 2014036520 A1 06-03-2014
WO 2011060206	A2	19-05-2011	AR 080564 A1 18-04-2012
			AU 2010319483 A1 31-05-2012
			BR 112012012160 A2 10-01-2017
			CA 2780935 A1 19-05-2011
			CN 102812045 A 05-12-2012
			CN 105999263 A 12-10-2016
			EP 2499162 A2 19-09-2012
			EP 2719708 A2 16-04-2014
			EP 2896632 A2 22-07-2015
			HK 1207651 A1 05-02-2016
			JP 5931736 B2 08-06-2016
			JP 2013510868 A 28-03-2013
			JP 2016128430 A 14-07-2016
			KR 20120114262 A 16-10-2012
			TW 201129378 A 01-09-2011
			US 2011229406 A1 22-09-2011
			US 2014017166 A1 16-01-2014
			US 2016222126 A1 04-08-2016
			UY 33034 A 31-05-2011
			WO 2011060206 A2 19-05-2011
WO 2014182970	A1	13-11-2014	AU 2014262566 A1 12-11-2015
			CA 2910945 A1 13-11-2014
			EP 2994164 A1 16-03-2016
			JP 2016520586 A 14-07-2016
			US 2016083480 A1 24-03-2016
			WO 2014182970 A1 13-11-2014