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(54) Title: ERBB-2 AND ERBB3 BINDING BISPECIFIC ANTIBODIES FOR USE IN THE TREATMENT F CELLS THAT HAVE AN NRG1 FUSION GENE

(57) Abstract: The invention relates to the field of antibodies. In particular it relates to the field of therapeutic (human) antibodies for the treatment of ErbB-2/ErbB-3 positive cells. More in particular it relates to treating of cells comprising an NRG1 fusion gene comprising at least a portion of the NRG1-gene fused to a sequence from a different chromosomal location.



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Title: ErbB-2 and ErbB3 binding bispecific antibodies for use in the treatment of cells that have an NRG1 fusion gene.

This application claims priority to EP Application No. 17164292.9, filed  
5 March 31, 2017 the content of which is hereby incorporated by reference.

The invention relates to the field of antibodies. In particular it relates to the field of therapeutic (human) antibodies for the treatment of a ErbB-2/ErbB-3 positive cell. More in particular it relates to treating tumors comprising an NRG1  
10 fusion gene comprising at least a portion of the NRG1-gene fused to a sequence from a different chromosomal location.

Neuregulin-1 (NRG1) has been proposed as a candidate oncogene and as a candidate tumor suppressor gene. It is likely to be involved in epithelial cancers,  
15 because it encodes ligands that can bind to the ErbB-family of receptors. To date, there are over 16 soluble and transmembrane proteins derived from the NRG1 gene. Proteolytic processing of the extra cellular domain of the transmembrane NRG1 isoforms release soluble factors. HRG1- $\beta$ 1 is one of the proteins encoded by the gene. It contains an Ig domain and an EGF-like domain that is necessary for  
20 direct binding to receptor tyrosine kinases ErbB-3 and ErbB-4. The NRG1 gene and the isoforms are known under a number of different aliases such as: Neuregulin 1; Pro-NRG1; HRGA; SMDF; HGL; GGF; NDF; NRG1 Intronic Transcript 2 (Non-Protein Coding); Heregulin, Alpha (45kD, ERBB2 P185-Activator); Acetylcholine Receptor-Inducing Activity; Pro-Neuregulin-1, Membrane-  
25 Bound Isoform; Sensory And Motor Neuron Derived Factor; Neu Differentiation Factor; Glial Growth Factor 2; NRG1-IT2; MSTP131; MST131; ARIA; GGF2; HRG1; and HRG. External Ids for NRG1 Gene are HGNC: 7997; Entrez Gene: 3084; Ensembl: ENSG00000157168; OMIM: 142445 and UniProtKB: Q02297.

Isoforms of NRG1 are made by alternative splicing, and include forms that  
30 are transmembrane, externally membrane bound, shed, secreted or intracellular (Falls, 2003; Hayes and Gullick, 2008). They bind to ErbB-3 or ErbB-4, which probably signal as heterodimers with ErbB-2 (HER2). Although the NRG1-encoded proteins are usually thought of as mitogens, they can also be powerfully

proapoptotic: in particular, expressing NRG1 in cells can cause apoptosis of the expressing cell (Weinstein et al., 1998).

The NRG1 gene has been identified as a potential cancer-critical gene in two, apparently contradictory, contexts. First, it is the prime candidate for the major tumor suppressor gene thought to be on chromosome 8p, the short arm of chromosome 8. Loss of chromosome 8p is one of the most frequent genomic events in epithelial cancers, including breast, colon, bladder and prostate. This has been shown successively by loss of heterozygosity, comparative genomic hybridization (CGH) and array-CGH studies (for references, see Birnbaum et al., 2003; Pole et al., 2006). The classical interpretation of this loss of chromosome 8p would be that there is a tumor suppressor gene there. Chromosome 8p losses in carcinoma cell lines were mapped using fluorescence-in situ hybridization and array-comparative genomic hybridization (array-CGH). It was found that most breaks were proximal to, or actually within NRG1, making NRG1 and genes immediately telomeric to NRG1 the prime candidates for such a tumor suppressor (Pole et al., 2006; Cooke et al., 2008). Second, NRG1 could be an oncogene because it seems to be the target of chromosome translocations in breast cancer (for review see Chua et al 2009).

In the present invention it was found that tumors with chromosome 8p modifications exhibit growth inhibition in response to exposure to a bispecific antibody that comprises a first antigen-binding site that can bind an extracellular part of ErbB-2 and a second antigen-binding site that can bind an extracellular part of ErbB-3.

Reference to any prior art in the specification is not an acknowledgement or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be combined with any other piece of prior art by a skilled person in the art.

## SUMMARY OF THE INVENTION

In one aspect, a method is provided for the treatment of an individual that has an ErbB-2 and ErbB-3 positive cell, the method comprising administering a bispecific antibody that comprises a first antigen-binding site that can bind an extracellular part of ErbB-2 and a second antigen-binding site that can bind an extracellular part of ErbB-3 to the individual in need thereof, the method characterized in that cell that comprises an NRG1 fusion gene comprising at least a portion of the NRG1-gene fused to a sequence from a different chromosomal location. Typically, the cell comprises an NRG1 fusion gene comprising at least the

3' end of the NRG1-gene fused to a 5' sequence from a different chromosomal location.

The said cell may be a cancer cell. The said cancer cell may be a cancer cell associated with an NRG1-fusion gene, such as a cancer cell driven by an NRG1-  
5 fusion.

In another aspect, a method is provided for the treatment of an individual that has an ErbB-2 and ErbB-3 positive tumor or is at risk of having said tumor, the method comprising administering a bispecific antibody that comprises a first antigen-binding site that can bind an extracellular part of ErbB-2 and a second  
10 antigen-binding site that can bind an extracellular part of ErbB-3 to the individual in need thereof, the method characterized in that cells of the tumor comprises an NRG1 fusion gene comprising a portion of the NRG1 fusion, such as the 3' end of the NRG1-gene, fused to a sequence, such as a 5' sequence, from a different chromosomal location.

15 An individual at risk of having an ErbB-2 and ErbB-3 positive tumor may be an individual that is in remission.

Preferably, the NRG1-fusion gene expresses a protein that comprises an NRG1 EGF-like domain. Preferably, the NRG-fusion is a fusion of NRG1 and a gene on human chromosome 8. Preferably, the gene on human chromosome 8  
20 encodes an excreted protein or a cellular membrane associated protein. Preferably, the NRG1 fusion gene is a fusion of the 3' end of the NRG1-gene with the 5' sequence of one of the genes selected from the group consisting of CD74; DOC4; TNFRSF10B; CLU; VAMP2; SLC3A2; RBPMS; WRN; SDC4; KIF13B; SLECA2; PDE7A; ATP1B1; CDK1; BMPR1B; MCPH1 and RAB2IL1.

25 Preferably, the cell is an epithelial cell. Preferably, the cell is a breast cancer cell, an ovarian cancer cell, a lung cancer cell, such as non-small cell lung cancer or a metastasis thereof.

Preferably, the tumor is of an epithelial origin. Preferably, the tumor is a breast cancer, ovarian cancer, lung cancer, or a metastasis thereof.

30 The cell may be a cancer cell, for example an ovarian cancer comprising, for example, the CLU-NRG1 fusion or the RAB2IL1-NRG1

The cell may be a cancer cell, for example a breast cancer cell comprising, for example, the DOC4-NRG1 fusion

The cell may be a cancer cell, for example a NSCLC (lung) cancer, such as the subtype termed invasive mucinous adenocarcinoma, comprising, for example, VAMP2-NRG1, RBPMS-NRG1, WRN-NRG1, SDC4-NRG1, SLEC3A2-NRG1, KIF13B-NRG1 or CD74-NRG1.

5            Preferably, the individual has undergone a therapy that targeted towards EGFR inhibition, preferably with an EGFR binding antibody, which is preferably cetuximab.

            Preferably, the method further comprises determining the ErbB-1 cell-surface receptor density; ErbB-2 cell-surface receptor density; ErbB-3 cell-surface  
10            receptor density; ErbB-4 cell-surface receptor density or a combination thereof on the cells of the tumor. Preferably, the cell or tumor has less than 400,000 ErbB-1 cell-surface receptors per cell, preferably less than 200,000 ErbB-1 cell-surface receptors per cell.

            Preferably, the method further comprises administering to the individual  
15            an ErbB-1 inhibitor, preferably cetuximab.

            Preferably in the methods disclosed herein, the ErbB-2/ErbB-3 positive cell or tumor has less than 50,000 ErbB-3 cell-surface receptors per cell.

            Preferably in the methods disclosed herein, the cell or cells of said tumor have a heregulin expression level that is greater than the heregulin expression  
20            level of MCF7 cells.

            As is clear to a skilled person the bispecific antibodies disclosed herein are also for the use in the preparation of a medicament and for the use in therapy, as disclosed herein.

            In particular, a bispecific antibody that comprises a first antigen-binding  
25            site that can bind an extracellular part of ErbB-2 and a second antigen-binding site that can bind an extracellular part of ErbB-3 for use in the treatment of an individual that has an ErbB-2 and ErbB-3 positive cell, which cell comprises an NRG1 fusion gene comprising at least the 3' end of the NRG1-gene fused to a 5' sequence from a different chromosomal location.

30            The said cell may be a cancer cell. The said cancer cell may be a cancer cell associated with an NRG1-fusion, such as a cancer cell driven by an NRG1-fusion.

            Also, the bispecific antibodies are for use in the treatment of an ErbB-2/ErbB-3 positive tumor, wherein the cells of the tumor comprise an NRG1 fusion

gene comprising the 3' end of the NRG1-gene fused to a 5' sequence from a different chromosomal location.

Preferably in the methods and uses disclosed herein, said first antigen-binding site binds domain I of ErbB-2 and said second antigen-binding site binds domain III of ErbB-3, preferably wherein the affinity of the first antigen-binding site for ErbB-2 is lower than the affinity of the second antigen-binding site for ErbB-3. Preferably wherein said bispecific antibody comprises

5 i) at least the CDR1, CDR2 and CDR3 sequences of an ErbB-2 specific heavy chain variable region selected from the group consisting of MF2926, MF2930, MF1849; MF2973, MF3004, MF3958, MF2971, MF3025, MF2916, MF3991, MF3031, MF2889, MF2913, MF1847, MF3001, MF3003 and MF1898 or wherein said antibody comprises CDR sequences that differ in at most 3 amino acids, preferably in at most 2 amino acids, preferably in at most 1 amino acid from the CDR1, CDR2 and CDR3 sequences of MF2926, MF2930, MF1849; MF2973, MF3004, MF3958,

10 MF2971, MF3025, MF2916, MF3991, MF3031, MF2889, MF2913, MF1847, MF3001, MF3003 or MF1898; and/or

ii) at least the CDR1, CDR2 and CDR3 sequences of an ErbB-3 specific heavy chain variable region selected from the group consisting of MF3178; MF3176; MF3163; MF3099; MF3307; MF6055; MF6056; MF6057; MF6058; MF6059; MF6060;

20 MF6061; MF6062; MF6063; MF6064; MF 6065; MF6066; MF6067; MF6068; MF6069; MF6070; MF6071; MF6072; MF6073 and MF6074, or wherein said antibody comprises CDR sequences that differ in at most 3 amino acids, preferably in at most 2 amino acids, preferably in at most 1 amino acid from the CDR1, CDR2 and CDR3 sequences of MF3178; MF3176; MF3163; MF3099; MF3307; MF6055;

25 MF6056; MF6057; MF6058; MF6059; MF6060; MF6061; MF6062; MF6063; MF6064; MF 6065; MF6066; MF6067; MF6068; MF6069; MF6070; MF6071; MF6072; MF6073 or MF6074. Preferably, the antibody comprises

i) an ErbB-2 specific heavy chain variable region sequence selected from the group consisting of the heavy chain variable region sequences of MF2926, MF2930,

30 MF1849; MF2973, MF3004, MF3958, MF2971, MF3025, MF2916, MF3991, MF3031, MF2889, MF2913, MF1847, MF3001, MF3003 and MF1898, or wherein said antibody comprises a heavy chain variable region sequence that differs in at most 15 amino acids from the heavy chain variable region sequences of MF2926,

MF2930, MF1849; MF2973, MF3004, MF3958, MF2971, MF3025, MF2916,  
MF3991, MF3031, MF2889, MF2913, MF1847, MF3001, MF3003 or MF1898;  
and/or

ii) an ErbB-3 specific heavy chain variable region sequence selected from the group  
5 consisting of the heavy chain variable region sequences of MF3178; MF3176;  
MF3163; MF3099; MF3307; MF6055; MF6056; MF6057; MF6058; MF6059;  
MF6060; MF6061; MF6062; MF6063; MF6064; MF 6065; MF6066; MF6067;  
MF6068; MF6069; MF6070; MF6071; MF6072; MF6073 and MF6074, or wherein  
10 said antibody comprises a heavy chain variable region sequence that differs in at  
most 15 amino acids from the heavy chain variable region sequences of MF3178;  
MF3176; MF3163; MF3099; MF3307; MF6055; MF6056; MF6057; MF6058;  
MF6059; MF6060; MF6061; MF6062; MF6063; MF6064; MF 6065; MF6066;  
MF6067; MF6068; MF6069; MF6070; MF6071; MF6072; MF6073 or MF6074.  
Preferably, the antibody comprises at least the CDR1, CDR2 and CDR3 sequences  
15 of the ErbB-2 specific heavy chain variable region MF3958 and the antibody  
comprises at least the CDR1, CDR2 and CDR3 sequences of the ErbB-3 specific  
heavy chain variable region MF3178. Preferably, the bispecific antibody comprises  
the “heavy chain for erbB-2 binding” as depicted in the Sequence listing part 1D  
and the “heavy chain for erbB-3 binding” as depicted in the Sequence listing part  
20 1D.

Preferably, the first antigen binding site and said second antigen binding  
site comprise a light chain variable region comprising the IgVKI-39 gene segment,  
most preferably the rearranged germline human kappa light chain IgVKI-  
39\*01/IGJKI\*01 or IgVκ1-39\*01/IGJκ5\*01. Preferably, the light chain variable  
25 region comprises a CDR1 having the sequence (RASQSISSYLN), a CDR2 having  
the sequence (AASSLQS), and a CDR3 having the sequence (QQSYSTPPT).

#### DETAILED DESCRIPTION OF THE INVENTION

An NRG1 fusion gene comprises at least a portion of the NRG1-gene fused  
30 to a sequence from a different chromosomal location. “At least a portion” indicates  
that the entire NRG-1 gene may be present in a fusion or a portion thereof. The  
fusion preferably has at least the coding sequence of exons 6, 7 and 8. Another way  
to define the NRG1 part in the NRG1-fusion gene is that it comprises the EGF-like

domain of NRG1. The at least a portion of the NRG1 gene may be fused to a sequence from a different chromosomal location such that the said sequence is located 5' or 3'to the at least a portion of the NRG1 gene.

Preferably, the 3' end of the NRG1-gene may be fused to a 5' sequence from  
5 a different chromosomal location. The NRG1-gene codes for the various isoforms of NRG1. Various isoforms and their expected function are described in Adelaide et al (2003). GGF and GGF2 isoforms contain a kringle-like sequence plus Ig and EGF-like domains; and the SMDF isoform shares only the EGF-like domain with other isoforms. The EGF-like domain is encoded by the 3' end of the gene. The EGF-like  
10 domain is present in all NRG1 fusion genes of the present invention. Fusions have been found wherein the 5' from different chromosomal location comprises an excretion signal and/or or a transmembrane domain of a cellular membrane protein with at least one extracellular domain. An example is the CD74-NRG1 fusion. The 5' sequence from different chromosomal location may also insert a sequence that  
15 activates transcription of NRG1, examples are promoters or enhancers. The 5' sequence is typically a sequence from a gene different than NRG1. The sequence can comprise a coding region, an expression regulatory sequence such as a promoter or an enhancer, or a combination thereof. The NRG-fusion comprises a 5' sequence from a different location can be from a different chromosome or from  
20 another part of chromosome 8. In a preferred embodiment the 5' sequence is from a gene on human chromosome 8.

The NRG1-gene, for example the 3' end of the NRG-1 gene, in the fusion preferably has at least the coding sequence of exons 6, 7 and 8. Another way to define the NRG1 part in the NRG1-fusion gene is that it comprises the EGF-like  
25 domain of NRG1. This domain is encoded by the 3' end of the NRG1 gene (exons 6-8) and is necessary for binding to ErbB-3. NRG1-fusions retain an in frame coding region for this EGF-like domain at the 3'end of the fusion. An EGF-like domain is a sequence of typically about thirty to forty amino-acid residues long of which the prototype is found in the sequence of epidermal growth factor (EGF) [PMID:  
30 2288911, PMID: 6334307, PMID: 1522591, PMID: 6607417, PMID: 3282918, PMID: 11498013]. It is known to be present, in a more or less conserved form, in a large number of other, mostly animal proteins. A common feature of EGF-like domains is that they are found in the extracellular domain of membrane-bound

proteins or in proteins known to be secreted (exception: prostaglandin G/H synthase). The EGF domain typically includes six cysteine residues which have been shown (in EGF) to be involved in disulphide bonds. The main structure is a two-stranded beta-sheet followed by a loop to a C-terminal short two-stranded  
5 sheet. Subdomains between the conserved cysteines vary in length.

The NRG1 fusion gene is preferably a fusion of the 3' end of the NRG1-gene with the 5' sequence of one of the genes selected from the group consisting of CD74; DOC4; TNFRSF10B; CLU; VAMP2; SLC3A2; RBPMS; WRN; SDC4; KIF13B; SLECA2; PDE7A; ATP1B1; CDK1; BMPR1B; MCPH1 and RAB2IL1.

10 The NRG1 fusion gene may be a fusion of at least a portion of the NRG1-gene with a sequence from a different chromosomal location located 3' thereto. Such a NRG1 fusion gene may be a fusion of at least a portion of the NRG1-gene with a sequence from a different chromosomal location CD74, STMN2, PMEPA1, PROSC or PSAP located 3' thereto. The receptors for all NRG1 isoforms are the  
15 ErbB family of tyrosine kinase transmembrane receptors. The family is also referred to as the human epidermal growth factor (EGF) receptor family (HER). The family has four members: ErbB (Erythroblastoma)-1, ErbB-2, ErbB-3 and ErbB-4. Epidermal growth factor (EGF) receptor (EGFR, ErbB1, or HER1). The receptors (reviewed in Yarden and Pines 2012) are widely expressed on epithelial  
20 cells. Upregulation of HER receptors or their ligands, such as heregulin (HRG) or epidermal growth factor (EGF), is a frequent event in human cancer (Wilson, Fridlyand et al. 2012). Overexpression of ErbB-1 and ErbB-2 in particular occurs in epithelial tumors and is associated with tumor invasion, metastasis, resistance to chemotherapy, and poor prognosis (Zhang, Berezov et al. 2007). In the normal  
25 breast, ErbB-3 has been shown to be important in the growth and differentiation of luminal epithelium. For instance, loss/inhibition of ErbB-3 results in selective expansion of the basal over the luminal epithelium (Balko, Miller et al. 2012). Binding of ligand to the extracellular domain of the RTKs induces receptor dimerization, both between the same (homodimerization) and different  
30 (heterodimerization) receptor subtypes. Dimerization can activate the intracellular tyrosine kinase domains, which undergo autophosphorylation and, in turn, can activate a number of downstream pro-proliferative signaling pathways, including those mediated by mitogen-activated protein kinases (MAPK) and the prosurvival

pathway Akt (reviewed in Yarden and Pines, 2012). No specific endogenous ligand has been identified for ErbB-2, which is therefore assumed to normally signal through heterodimerization (Sergina, Rausch et al. 2007). ErbB-3 can be activated by engagement of its ligands. These ligands include but are not limited to  
5 neuregulin (NRG) and heregulin (HRG).

ErbB-1 is known under various synonyms, the most common of which is EGFR. EGFR has an extracellular domain (ECD) that is composed of four sub-  
domains, two of which are involved in ligand binding and two of which are involved  
10 in homo-dimerisation and hetero-dimerisation. EGFR integrates extracellular signals from a variety of ligands to yield diverse intracellular responses. The major signal transduction pathway activated by EGFR is composed of the Ras-mitogen-activated protein kinase (MAPK) mitogenic signalling cascade. Activation of this pathway is initiated by the recruitment of Grb2 to tyrosine phosphorylated EGFR.  
15 This leads to activation of Ras through the Grb2-bound Ras-guanine nucleotide exchange factor Son of Sevenless (SOS). In addition, the PI3-kinase-Akt signal transduction pathway is also activated by EGFR, although this activation is much stronger in case there is co-expression of ErbB-3 (HER3). The EGFR is implicated in several human epithelial malignancies, notably cancers of the breast, bladder,  
20 non-small cell lung cancer lung, colon, ovarian head and neck and brain. Activating mutations in the gene have been found, as well as over-expression of the receptor and of its ligands, giving rise to autocrine activation loops. This RTK has therefore been extensively used as target for cancer therapy. Both small-molecule inhibitors targeting the RTK and monoclonal antibodies (mAbs) directed to the extracellular  
25 ligand-binding domains have been developed and have shown hitherto several clinical successes, albeit mostly for a select group of patients. The database accession number for the human EGFR protein and the gene encoding it is (GenBank NM\_005228.3). This accession number is primarily given to provide a further method of identification of EGFR protein as a target, the actual sequence of the EGFR protein bound by an antibody may vary, for instance because of a  
30 mutation in the encoding gene such as those occurring in some cancers or the like.

The words cancer and tumor are used herein typically both refer to cancer, unless otherwise specifically stated.

Where reference herein is made to EGFR, the reference refers to human  
5 EGFR unless otherwise stated. The antigen-binding site that binds EGFR, binds EGFR and a variety of variants thereof such as those expressed on some EGFR positive tumors.

The term 'ErbB-3' as used herein refers to the protein that in humans is  
10 encoded by the ERBB3 gene. Alternative names for the gene or protein are HER3; LCCS2; MDA-BF-1; c-ErbB-3; c-ErbB3; ErbB3-S; p180-ErbB3; p45-sErbB3; and p85-sErbB3. Where reference is made herein to ErbB-3, the reference refers to human ErbB-3. An antibody comprising an antigen-binding site that binds ErbB-3, binds human ErbB-3. The ErbB-3 antigen-binding site may, due to sequence and  
15 tertiary structure similarity between human and other mammalian orthologs, also bind such an ortholog but not necessarily so. Database accession numbers for the human ErbB-3 protein and the gene encoding it are (NP\_001005915.1, NP\_001973.2, NC\_000012.11, NC\_018923.2, NT\_029419.12). The accession numbers are primarily given to provide a further method of identification of ErbB-3  
20 as a target, the actual sequence of the ErbB-3 protein bound by an antibody may vary, for instance because of a mutation in the encoding gene such as those occurring in some cancers or the like. The ErbB-3 antigen binding site binds ErbB-3 and a variety of variants thereof, such as those expressed by some ErbB-3 positive tumor cells. The antigen-binding site that binds ErbB-3 preferably binds  
25 domain III of ErbB-3.

The term 'ErbB-2' as used herein refers to the protein that in humans is encoded by the ERBB2 gene. Alternative names for the gene or protein include CD340; HER-2; HER-2/neu; MLN 19; NEU; NGL; TKR1. The ERBB2 gene is  
30 frequently called HER2 (from human epidermal growth factor receptor 2). Where reference is made herein to ErbB-2, the reference refers to human ErbB-2. An antibody comprising an antigen-binding site that binds ErbB-2, binds human ErbB-2. The ErbB-2 antigen-binding site may, due to sequence and tertiary

structure similarity between human and other mammalian orthologs, also bind such an ortholog but not necessarily so. Database accession numbers for the human ErbB-2 protein and the gene encoding it are (NP\_001005862.1, NP\_004439.2 NC\_000017.10 NT\_010783.15 NC\_018928.2). The accession numbers are  
 5 primarily given to provide a further method of identification of ErbB-2 as a target, the actual sequence of the ErbB-2 protein bound the antibody may vary, for instance because of a mutation in the encoding gene such as those occurring in some cancers or the like. The ErbB-2 antigen binding site binds ErbB-2 and a variety of variants thereof, such as those expressed by some ErbB-2 positive tumor  
 10 cells. The antigen-binding site that binds ErbB-2 preferably binds domain I of ErbB-2.

CD74 is known under number of aliases. Some of these are CD74 Molecule; CD74 Antigen (Invariant Polypeptide Of Major Histocompatibility Complex, Class  
 15 II Antigen-Associated); CD74 Molecule, Major Histocompatibility Complex, Class II Invariant Chain; HLA-DR Antigens-Associated Invariant Chain; Gamma Chain Of Class II Antigens; Ia-Associated Invariant Chain; MHC HLA-DR Gamma Chain; HLA-DR-Gamma; DHLAG; P33; HLA Class II Histocompatibility Antigen Gamma Chain; Ia Antigen-Associated Invariant Chain; Ia-GAMMA and HLADG. External  
 20 Ids for CD74 are HGNC: 1697; Entrez Gene: 972; Ensembl: ENSG00000019582; OMIM: 142790 and UniProtKB: P04233.

DOC4 or Teneurin Transmembrane Protein 4 (TENM4) is known under a number of different names such as Protein Odd Oz/Ten-M Homolog 4; Tenascin-  
 25 M4; Ten-M4; Ten-4; ODZ4; TNM4; Odz, Odd Oz/Ten-M Homolog 4 (Drosophila); Odz, Odd Oz/Ten-M Homolog 4; Teneurin-4; KIAA1302; Doc4; and ETM5. External Ids for DOC4 are HGNC: 29945; Entrez Gene: 26011; Ensembl: ENSG00000149256; OMIM: 610084 and UniProtKB: Q6N022.

30 TNFRSF10B or TNF Receptor Superfamily Member 10b is known under a number of different names Tumor Necrosis Factor Receptor Superfamily, Member 10b; TNF-Related Apoptosis-Inducing Ligand Receptor 2; Death Receptor 5; TRAIL-R2; TRAILR2; KILLER; TRICK2; ZTNFR9; DR5; P53-Regulated DNA

Damage-Inducible Cell Death Receptor(Killer); Tumor Necrosis Factor Receptor Superfamily Member 10B; Tumor Necrosis Factor Receptor-Like Protein ZTNFR9; Death Domain Containing Receptor For TRAIL/Apo-2L; poptosis Inducing Protein TRICK2A/2B; Apoptosis Inducing Receptor TRAIL-R2; Cytotoxic TRAIL Receptor-  
5 2; Fas-Like Protein; TRAIL Receptor 2; CD262 Antigen; KILLER/DR5; TRICK2A; TRICK2B; TRICKB; and CD262. External Ids for TNFRSF10B are HGNC: 11905; Entrez Gene: 8795; Ensembl: ENSG00000120889; OMIM: 603612; and UniProtKB: O14763.

10 The CLU gene or Clusterin is known under a number of different names such as Testosterone-Repressed Prostate Message 2; Apolipoprotein J; Complement-Associated Protein SP-40,40; Complement Cytolysis Inhibitor; Complement Lysis Inhibitor; Sulfated Glycoprotein 2; Ku70-Binding Protein 1; NA1/NA2; TRPM-2; APO-J; APOJ; KUB1; CLI; Clusterin (Complement Lysis  
15 Inhibitor, SP-40,40, Sulfated Glycoprotein 2, Testosterone-Repressed Prostate Message 2, Apolipoprotein J); Aging-Associated Gene 4 Protein; Aging-Associated Protein 4; SGP-2; SP-40; TRPM2; AAG4; CLU1; CLU2; and SGP2. External Ids for CLU are HGNC: 2095; Entrez Gene: 1191; Ensembl: ENSG00000120885; OMIM: 185430; and UniProtKB: P10909.

20

VAMP2 or Vesicle Associated Membrane Protein 2 is known under a number of different names such as synaptobrevin 2; SYB2; Vesicle-Associated Membrane Protein 2; and Synaptobrevin-2. External Ids for VAMP2 are HGNC: 12643; Entrez Gene: 6844; Ensembl: ENSG00000220205; OMIM: 185881; and  
25 UniProtKB: P63027.

SLCA3A2 or Solute Carrier Family 3 Member 2 is known under a number of different names such as Lymphocyte Activation Antigen 4F2 Large Subunit; Solute Carrier Family 3 (Activators Of Dibasic And Neutral Amino Acid Transport),  
30 Member 2; Antigen Identified By Monoclonal Antibodies 4F2, TRA1.10, TROP4, And T43; Solute Carrier Family 3 (Amino Acid Transporter Heavy Chain), Member 2; 4F2 Cell-Surface Antigen Heavy Chain; CD98 Heavy Chain; 4F2HC; MDU1; Antigen Defined By Monoclonal Antibody 4F2, Heavy Chain; Antigen Defined By

Monoclonal Antibody 4F2; 4F2 Heavy Chain Antigen; 4F2 Heavy Chain; CD98 Antigen; CD98HC; 4T2HC; NACAE; CD98 and 4F2. External Ids for SLC3A2 are HGNC: 11026; Entrez Gene: 6520; Ensembl: ENSG00000168003; OMIM: 158070; and UniProtKB: P08195.

5

RBPM5 or RNA Binding Protein With Multiple Splicing is known under a number of different names such as RNA Binding Protein With Multiple Splicing; Heart And RRM Expressed Sequence; HERMES; RNA-Binding Protein With Multiple Splicing; and RBP-MS. External Ids for RBPM5 are HGNC: 19097; 10 Entrez Gene: 11030; Ensembl: ENSG00000157110; OMIM: 601558; and UniProtKB: Q93062.

WRN or Werner Syndrome RecQ Like Helicase is known under a number of different names such as Werner Syndrome RecQ Like Helicase; DNA Helicase, 15 RecQ-Like Type 3; RecQ Protein-Like 2; Exonuclease WRN; RECQL2; RECQ3; Werner Syndrome ATP-Dependent Helicase; Werner Syndrome, RecQ Helicase-Like; Werner Syndrome; EC 3.6.4.12; EC 3.1.-.-; EC 3.6.1; and RECQL3. External Ids for WRN are HGNC: 12791; Entrez Gene: 7486; Ensembl: ENSG00000165392; OMIM: 604611 and UniProtKB: Q14191.

20

SDC4 or Syndecan 4 is known under a number of different names such as Syndecan 4 (Amphiglycan, Ryudocan); Syndecan Proteoglycan 4; Ryudocan Core Protein; Amphiglycan; SYND4; Ryudocan Amphiglycan; and Syndecan-4. External Ids for SDC4 are HGNC: 10661; Entrez Gene: 6385; Ensembl: ENSG00000124145; 25 OMIM: 600017; and UniProtKB: P31431.

Various NRG1 fusion genes are described in Dhanasekaran et al (2014).

The invention provides methods of an individual that has an ErbB-2 and 30 ErbB-3 positive cell or tumor. Alternatively, the individual may be at risk of having a tumor. The method comprises administering a bispecific antibody that comprises a first antigen-binding site that can bind an extracellular part of ErbB-2 and a second antigen-binding site that can bind an extracellular part of ErbB-3 to

the individual in need thereof. The method is characterized in that the cell or cells of the tumor comprises an NRG1 fusion gene comprising the 3' end of the NRG1-gene fused to a 5' sequence from a different chromosomal location.

The cell may be a cancer cell. Said cancer cell may be a cancer cell  
5 associated with an NRG1-fusion, for example a cancer cell driven by an NRG1-fusion.

Antigen-binding sites in an antibody are typically present in the variable domains. The variable domains comprise a heavy chain variable region and a light  
10 chain variable region.

The individual has preferably undergone a therapy that targeted towards EGFR inhibition, preferably with an EGFR binding antibody, which is preferably  
15 cetuximab.

A method of treatment of the invention preferably further comprises determining the number of ErbB-1 cell-surface receptor; ErbB-2 cell-surface  
20 receptor; ErbB-3 cell-surface receptor; ErbB-4 cell-surface receptor or a combination thereof on the cell or cells of the tumor.

A method of treatment of the invention preferably further comprises determining whether the cell comprises an NRG1-fusion or whether the tumor  
25 comprises cells with an NRG1-fusion. This can for instance be done on cells of a biopsy. Various methods are available and many are known in the art. Known that region wherein the chromosome break occurs in the case of NRG1-fusions it is routine for the skilled person to determine whether a tumor comprises such an NRG1-fusion. One way is by means of PCR-amplification with primers that span the  
30 junction in the NRG1 fusion. This can easily be implemented for NRG1-fusions that are known to occur. New fusions can also be detected easily. A suitable way is for instance by junction cloning techniques used to find for instance the integration site of retroviral genomes. A suitable method is by means of LAM-PCR see Schmidt et al Nature Methods 4, 1051 - 1057 (2007) doi:10.1038/nmeth1103 and specific references to the LAM-technology therein.

A method of treatment of the invention is preferably characterized in that the cell or tumor has less than 400,000 ErbB-1 cell-surface receptors per cell, preferably less than 200.000 ErbB-1 cell-surface receptors per cell.

5 In a preferred embodiment the method of treatment of the invention further comprises administering to the individual an ErbB-1 inhibitor, preferably cetuximab.

The method of treatments as defined herein can also be defined as a  
10 compound or combination of compounds for use in the treatment of. Suitable combination of compounds are a bispecific antibody as defined herein and an ErbB-1 inhibitor.

To establish whether a cell or tumor is positive for ErbB-2 and ErbB-3 the  
15 skilled person can for instance determine the ErbB-2 and ErbB-3 amplification and/or staining in immunohistochemistry. At least 10% tumor cells in a biopsy should be positive for both ErbB-2 and for ErbB-3. The biopsy can also contain 20%, 30% 40% 50% 60% 70% or more positive cells. ErbB-1 positive tumors can be similarly identified.

20 Preferably said positive cancer is a breast cancer, such as early-stage breast cancer. However, the invention can be applied to a wide range of ErbB-2, ErbB-3 or ErbB-2/ErbB-3 positive cancers, like gastric cancer, colorectal cancer, colon cancer, gastro-esophageal cancer, esophageal cancer, endometrial cancer, ovarian cancer, breast cancer, liver cancer, lung cancer, including non-small cell lung cancer, clear  
25 cell sarcoma, salivary gland cancer, head and neck cancer, brain cancer, bladder cancer, pancreatic cancer, prostate cancer, kidney cancer, skin cancer, melanoma, and the like. The cell is preferably an epithelial cell. Alternatively, the cell or tumor is preferably a cell or tumor of an epithelial origin. In a preferred embodiment the cell or tumor is a breast cancer, an ovarian cancer, a lung cancer,  
30 or a metastasis thereof. Preferably the tumor is of epithelial origin. Preferably, the tumor is a breast cancer, an ovarian cancer, a lung cancer, or a metastasis thereof.

Patients with ErbB 2 positive cells or tumor cells can be classified based on the number of ErbB-2 receptors on the tumor cell surface. Tumors with more than

1,000,000 ErbB-2 receptors on their cell surface are typically classified as ErbB-2 [+++], those with between 150,000 to 1,000,000 are classified as ErbB-2 [++], and those with less than 150,000 are classified as ErbB-2[+]. Preferably, the patient is classified as ErbB-2[++] or ErbB-2 [+++]. Preferably, the ErbB-2/ErbB-3 positive  
5 tumor has at least 1,000,000 ErbB-2 cell-surface receptors per cell.

Preferably, methods are provided in which the ErbB-2/ErbB-3 positive cell or tumor has at least 150,000 ErbB-2 cell-surface receptors per cell and less than 50,000 ErbB-3 cell-surface receptors per cell. Preferably, methods are provided in which the ErbB-2/ErbB-3 positive cell or tumor has at least 1,00,000 ErbB-2 cell-  
10 surface receptors per cell and less than 50,000 ErbB-3 cell-surface receptors per cell.

In some embodiments, the methods disclosed herein are advantageous in that specific patient populations are first determined based on, e.g., the ErbB-1, ErbB-2, and/or ErbB-3 cell-surface receptor density. Accordingly, the methods  
15 disclosed herein preferably comprise determining the ErbB-1 cell-surface receptor density, ErbB-2 cell-surface receptor density, ErbB-3 cell-surface receptor density and/or ErbB-4 cell-surface receptor density for said cell or tumor. As used herein, the term cell-surface receptors density refers to the number of receptors present at the cell-surface per cell.

20 Preferably, the methods disclosed herein further comprise determining the ErbB-2 cell-surface receptor density for said cell or tumor. Patients may be classified using immunohistochemistry or fluorescence *in situ* hybridization. The HercepTest™ and/or HER2 FISH (pharm Dx™), marketed both by Dako Denmark A/S, and/or using a HERmark® assay, marketed by Monogram Biosciences are  
25 examples of suitable assays for determining ErbB-2 or ErbB-3 cell surface receptor density. Other methods for determining the ErbB-2 receptor cell density are well-known to a skilled person. In vivo methods for determining ErbB-2 are also known, see, e.g., Chernomoridik et al. Mol Imaging. 2010 Aug; 9(4): 192–200 and Ardeshirpour et al. Technol Cancer Res Treat. 2014 Oct; 13(5): 427–434.

30 Preferably, the methods disclosed herein further comprise determining the ErbB-2 cell-surface receptor density for said cell or tumor. Such methods are known to a skilled person (see, e.g., van der Woning and van Zoelen Biochem Biophys Res Commun. 2009 Jan 9;378(2):285-9). Preferably, the methods disclosed herein further

comprise determining the ErbB-1 cell-surface receptor density for said cell or tumor. Such methods are known to a skilled person (see, e.g., EGFR pharmDx™Kit (Dako)) and McDonagh et al. *Mol Cancer Ther* 2012; 11:582). Similar methods can be used to determine ErbB-4 cell-surface receptor density.

5 In some embodiments, the ErbB-1, ErbB-2, ErbB-3, and ErbB-4 cell-surface receptor densities are determined by FACS analysis on biopsied tumor cells.

Preferably, the cells of the ErbB-2/ErbB-3 positive cell or tumor have relatively high levels of heregulin expression. Heregulin is a growth factor that is involved in growth of ErbB 3 positive cell or tumor cells. Typically, when the cell or  
10 tumor cells express high levels of heregulin (referred to as heregulin stress), currently known therapies like trastuzumab, pertuzumab and lapatinib are no longer capable of inhibiting cell or tumor growth. This phenomenon is called heregulin resistance. In particular, the heregulin expression level that is greater than the heregulin expression level of MCF7 cells. Heregulin expression levels are  
15 for instance measured using qPCR with cell or tumor RNA (such as for instance described in Shames et al. *PLOS ONE*, February 2013, Vol.8, Issue 2, pp 1-10 and in Yonesaka et al., *Sci.transl.Med.*, Vol.3, Issue 99 (2011); pp1-11), or using protein detection methods, like for instance ELISA, preferably using blood, plasma or serum samples (such as for instance described in Yonesaka et al., *Sci.transl.Med.*,  
20 Vol.3, Issue 99 (2011); pp1-11).

High heregulin levels are typically present during the formation of metastases (i.e. the migration, invasion, growth and/or differentiation of a cell or tumor cells or tumor initiating cells). Typically, tumor initiating cells are identified based on stem cell markers such as for instance CD44, CD24, CD133 and/or  
25 ALDH1. These processes can therefore barely be counteracted with currently known therapies like trastuzumab and pertuzumab. The bispecific antibodies disclosed herein are capable of counteracting the formation of metastases in subjects having cells tumors that comprise an NRG1 fusion gene comprising the 3' end of the NRG1-gene fused to a 5' sequence from a different chromosomal location.

30 Further provided is therefore a method for counteracting the formation of a metastasis in a subject having a ErbB-2, ErbB-3 or ErbB-2/ErbB-3 positive cell or tumor, wherein said ErbB-2, ErbB-3 or ErbB-2/ErbB-3 positive cell or tumor cell has a heregulin expression level that is at least 60%, preferably at least 70%, more

preferably at least 80%, more preferably at least 85%, more preferably at least 90% or 95% of the heregulin expression level of BXPC3 or MCF7 cells, comprising administering to the subject a bispecific antibody comprising a first antigen-binding site that binds ErbB-2 and a second antigen-binding site that binds ErbB-3. Also provided is a bispecific antibody comprising a first antigen-binding site that binds ErbB-2 and a second antigen-binding site that binds ErbB-3 for use in the treatment or prevention of the formation of metastases, wherein said ErbB-2, ErbB-3 or ErbB-2/ErbB-3 positive cell or tumor cell has a heregulin expression level that is at least 60%, preferably at least 70%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90% or 95% of the heregulin expression level of BXPC3 or MCF7 cells. Further provided is a use of a bispecific antibody according to the invention for the preparation of a medicament for the treatment or prevention of the formation of metastases, wherein said ErbB-2, ErbB-3 or ErbB-2/ErbB-3 positive cell or tumor cell has a heregulin expression level that is at least 60%, preferably at least 70%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90% or 95% of the heregulin expression level of BXPC3 or MCF7 cells.

The subject is preferably a human subject. The subject is preferably a subject eligible for monoclonal antibody therapy using an ErbB-2 specific antibody such as trastuzumab.

The amount of bispecific to be administered to a patient is typically in the therapeutic window, meaning that a sufficient quantity is used for obtaining a therapeutic effect, while the amount does not exceed a threshold value leading to an unacceptable extent of side-effects. The lower the amount of antibody needed for obtaining a desired therapeutic effect, the larger the therapeutic window will typically be. The selected dosage level will depend upon a variety of factors including 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, 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 dosage can be in the range of the dosing regime for trastuzumab or lower.

The bispecific antibodies can be formulated as a pharmaceutical composition comprising a pharmaceutically acceptable carrier, diluent, or excipient, and additional, optional, active agents. The antibodies and compositions comprising the antibodies can be administered by any route including parenteral, enteral, and  
5 topical administration. Parenteral administration is usually by injection, and includes, e.g., intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracerebro spinal, intratumoral, and  
10 intrasternal injection and infusion.

In preferred embodiments, an ErbB-1 inhibitor can be combined with treatment with the bispecific antibodies disclosed herein. The ErbB-1 inhibitor can be administered simultaneously or sequentially with the bispecific antibody. Treatment with the ErbB-1 inhibitor can be separated by several minutes, hours,  
15 or days from the treatment with the bispecific antibody. Preferably, the ErbB-2/ErbB3 cell or tumor is also positive for ErbB1. Preferably, the combination treatment is suitable for ErbB-2/ErbB3 cells or tumors having more than 5,000 surface receptors per cell, preferably at least 20,000 surface receptors per cell, more preferably more than 50,000 surface receptors per cell.

20 Suitable ErbB-1 inhibitors are known in the art and refer to compounds that inhibit at least one biological activity of ErbB-1 (EGFR), in particular a compound that decreases the expression or signaling activity of ErbB-1. Preferred ErbB-1 inhibitors bind to the extracellular binding site of the tyrosine kinase receptor molecule and block binding of the natural ligands, such as EGF. Such  
25 inhibitors include antibodies, antibody portions, and peptides comprising epitopes that target this extracellular EGF receptor binding domain. Preferably, the ErbB-1 inhibitor is an anti-ErbB-1 antibody, preferably selected from cetuximab, matuzumab, necitumumab, nimotuzumab, panitumumab, or zalutumumab. The invention is further related to ErbB-1 inhibitors which can bind or interact with  
30 the intracellular phosphorylation site or domain of the tyrosine kinase receptor molecule, such preventing or decreasing phosphorylation by tyrosine kinase. This can be achieved by small (chemical) molecule drugs. Preferred inhibitors include afatinib, erlotinib, gefitinib, lapatinib, osimertinib, and neratinib.

The disclosure provides bispecific antibodies for use in the methods and treatments described herein. Suitable bispecific antibodies comprise a first antigen-binding site that binds ErbB-2 and a second antigen-binding site that binds ErbB-3, wherein the bispecific antibody reduces or can reduce a ligand-induced receptor function of ErbB-3 on an ErbB-2 and ErbB-3 positive cell. Preferred antibodies and their preparation are disclosed in WO 2015/130173, which is hereby incorporated by reference. The examples in WO 2015/130173 further describe a number of properties of the antibodies, such as ligand binding and epitope mapping.

As used herein, the term "antigen-binding site" refers to a site derived from and preferably as present on a bispecific antibody which is capable of binding to antigen. An unmodified antigen-binding site is typically formed by and present in the variable domain of the antibody. The variable domain contains said antigen-binding site. A variable domain that binds an antigen is a variable domain comprising an antigen-binding site that binds the antigen.

In one embodiment an antibody variable domain comprises a heavy chain variable region (VH) and a light chain variable region (VL). The antigen-binding site can be present in the combined VH/VL variable domain, or in only the VH region or only the VL region. When the antigen-binding site is present in only one of the two regions of the variable domain, the counterpart variable region can contribute to the folding and/or stability of the binding variable region, but does not significantly contribute to the binding of the antigen itself.

As used herein, antigen-binding refers to the typical binding capacity of an antibody to its antigen. An antibody comprising an antigen-binding site that binds to ErbB-2, binds to ErbB-2 and, under otherwise identical conditions, at least 100-fold lower to the homologous receptors ErbB-1 and ErbB-4 of the same species. An antibody comprising an antigen-binding site that binds to ErbB-3, binds to ErbB-3 and, under otherwise identical conditions, not to the homologous receptors ErbB-1 and ErbB-4 of the same species. Considering that the ErbB-family is a family of cell surface receptors, the binding is typically assessed on cells that express the receptor(s). Binding of an antibody to an antigen can be assessed in various ways. One way is to incubate the antibody with the antigen (preferably cells expressing the antigen), removing unbound antibody (preferably by a wash step) and detecting bound antibody by means of a labeled antibody that binds to the bound antibody.

Antigen binding by an antibody is typically mediated through the complementarity regions of the antibody and the specific three-dimensional structure of both the antigen and the variable domain allowing these two structures to bind together with precision (an interaction similar to a lock and key), as opposed to random, non-specific sticking of antibodies. As an antibody typically recognizes an epitope of an antigen, and as such epitope may be present in other compounds as well, antibodies according to the present invention that bind ErbB-2 and/or ErbB-3 may recognize other proteins as well, if such other compounds contain the same epitope. Hence, the term “binding” does not exclude binding of the antibodies to another protein or protein(s) that contain the same epitope. Such other protein(s) is preferably not a human protein. An ErbB-2 antigen-binding site and an ErbB-3 antigen-binding site as defined herein typically do not bind to other proteins on the membrane of cells in a post-natal, preferably adult human. A bispecific antibody as disclosed herein is typically capable of binding ErbB-2 and ErbB-3 with a binding affinity of at least  $1 \times 10^{-6}$  M, as outlined in more detail below.

The term “interferes with binding” as used herein means that the antibody is directed to an epitope on ErbB-3 and the antibody competes with ligand for binding to ErbB-3. The antibody may diminish ligand binding, displace ligand when this is already bound to ErbB-3 or it may, for instance through steric hindrance, at least partially prevent that ligand can bind to ErbB-3.

The term “antibody” as used herein means a proteinaceous molecule, preferably belonging to the immunoglobulin class of proteins, containing one or more variable domains that bind an epitope on an antigen, where such domains are derived from or share sequence homology with the variable domain of an antibody. Antibodies for therapeutic use are preferably as close to natural antibodies of the subject to be treated as possible (for instance human antibodies for human subjects). Antibody binding can be expressed in terms of specificity and affinity. The specificity determines which antigen or epitope thereof is specifically bound by the binding domain. The affinity is a measure for the strength of binding to a particular antigen or epitope. Specific binding, is defined as binding with affinities (KD) of at least  $1 \times 10^{-6}$  M, more preferably  $1 \times 10^{-7}$  M, more preferably higher than  $1 \times 10^{-9}$  M. Typically, antibodies for therapeutic applications have affinities of

up to  $1 \times 10^{-10}$  M or higher. Antibodies such the bispecific antibodies of the present invention comprise the constant domains (Fc part) of a natural antibody. An antibody of the invention is typically a bispecific full length antibody, preferably of the human IgG subclass. Preferably, an antibody as disclosed herein is of the human IgG1 subclass. Such antibodies have good ADCC properties, have favorable half life upon in vivo administration to humans and CH3 engineering technology exists that can provide for modified heavy chains that preferentially form heterodimers over homodimers upon co-expression in clonal cells.

An antibody as disclosed herein is preferably a "full length" antibody. The term 'full length' is defined as comprising an essentially complete antibody, which however does not necessarily have all functions of an intact antibody. For the avoidance of doubt, a full length antibody contains two heavy and two light chains. Each chain contains constant (C) and variable (V) regions, which can be broken down into domains designated CH1, CH2, CH3, VH, and CL, VL. An antibody binds to antigen via the variable domains contained in the Fab portion, and after binding can interact with molecules and cells of the immune system through the constant domains, mostly through the Fc portion. The terms 'variable domain', 'VH/VL pair', 'VH/VL' are used herein interchangeably. Full length antibodies according to the invention encompass antibodies wherein mutations may be present that provide desired characteristics. Such mutations should not be deletions of substantial portions of any of the regions. However, antibodies wherein one or several amino acid residues are deleted, without essentially altering the binding characteristics of the resulting antibody are embraced within the term "full length antibody". For instance, an IgG antibody can have 1-20 amino acid residue insertions, deletions or a combination thereof in the constant region. For instance, ADCC activity of an antibody can be improved when the antibody itself has a low ADCC activity, by slightly modifying the constant region of the antibody (Junttila, T. T., K. Parsons, et al. (2010). "Superior In vivo Efficacy of Afucosylated Trastuzumab in the Treatment of HER2-Amplified Breast Cancer." *Cancer Research* 70(11): 4481-4489)

Full length IgG antibodies are preferred because of their favourable half life and the need to stay as close to fully autologous (human) molecules for reasons of immunogenicity. An antibody as disclosed herein is preferably a bispecific IgG

antibody, preferably a bispecific full length IgG1 antibody. IgG1 is favoured based on its long circulatory half life in man. In order to prevent any immunogenicity in humans it is preferred that the bispecific IgG antibody is a human IgG1.

The term 'bispecific' (bs) means that one part of the antibody (as defined  
5 above) binds to one epitope on an antigen whereas a second part binds to a different epitope. The different epitope is typically present on a different antigen. The first and second antigens are in fact two different proteins. A preferred bispecific antibody is an antibody that comprises parts of two different monoclonal antibodies and consequently binds to two different types of antigen. One arm of the  
10 bispecific antibody typically contains the variable domain of one antibody and the other arm contains the variable domain of another antibody. The heavy chain variable regions of the bispecific antibody are typically different from each other, whereas the light chain variable regions are preferably the same. A bispecific antibody wherein the different heavy chain variable regions are associated with the  
15 same, or a common, light chain is also referred to as a bispecific antibody with a common light chain.

Preferred bispecific antibodies can be obtained by co-expression of two different heavy chains and a common light chain in a single cell. When wildtype CH3 domains are used, co-expression of two different heavy chains and a common  
20 light chain will result in three different species, AA, AB and BB. To increase the percentage of the desired bispecific product (AB) CH3 engineering can be employed, or in other words, one can use heavy chains with compatible heterodimerization domains, as defined hereunder.

The term 'compatible heterodimerization domains' as used herein refers to  
25 protein domains that are engineered such that engineered domain A' will preferentially form heterodimers with engineered domain B' and vice versa, whereas homodimerization between A'-A' and B'-B' is diminished.

The term 'common light chain' refers to light chains which may be identical or have some amino acid sequence differences while the binding specificity of the  
30 full length antibody is not affected. It is for instance possible, to prepare or find light chains that are not identical but still functionally equivalent, e.g., by introducing and testing conservative amino acid changes, changes of amino acids in regions that do not or only partly contribute to binding specificity when paired with

the heavy chain, and the like. The terms 'common light chain', 'common VL', 'single light chain', 'single VL', with or without the addition of the term 'rearranged' are all used herein interchangeably.

5           A common light chain (variable region) preferably has a germline sequence. A preferred germline sequence is a light chain variable region that is frequently used in the human repertoire and has good thermodynamic stability, yield and solubility. In a preferred embodiment the light chain comprises a light chain region comprising the amino acid sequence of an O12 / IgV $\kappa$ 1-39\*01 gene segment as  
10 depicted in the Sequences 1C "Common light chain IGKV1-39/jk1" with 0-10, preferably 0-5 amino acid insertions, deletions, substitutions, additions or a combination thereof. IgV $\kappa$ 1-39 is short for Immunoglobulin Variable Kappa 1-39 Gene. The gene is also known as Immunoglobulin Kappa Variable 1-39; IGKV139; IGKV1-39; O12a or O12. External Ids for the gene are HGNC: 5740; Entrez Gene:  
15 28930; Ensembl: ENSG00000242371. The variable region of IGKV1-39 is listed in the Sequences 1C. The V-region can be combined with one of five J-regions. Sequences 1C describe two preferred sequences for IgV $\kappa$ 1-39 in combination with a J-region. The joined sequences are indicated as IGKV1-39/jk1 and IGKV1-39/jk5; alternative names are IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 5\*01  
20 (nomenclature according to the IMGT database worldwide web at imgt.org).

It is preferred that the O12 / IgV $\kappa$ 1-39\*01 comprising light chain variable region is a germline sequence. It is further preferred that the IGJ $\kappa$ 1\*01 or /IGJ $\kappa$ 5\*01 comprising light chain variable region is a germline sequence. In a preferred embodiment, the IGKV1-39/jk1 or IGKV1-39/jk5 light chain variable  
25 regions are germline sequences.

In a preferred embodiment the light chain variable region comprises a germline O12 / IgV $\kappa$ 1-39\*01. In a preferred embodiment the light chain variable region comprises the kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 5\*01. In a preferred embodiment a IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01. The light  
30 chain variable region preferably comprises a germline kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or germline kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 5\*01, preferably a germline IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01.

Obviously, those of skill in the art will recognize that “common” also refers to functional equivalents of the light chain of which the amino acid sequence is not identical. Many variants of said light chain exist wherein mutations (deletions, substitutions, additions) are present that do not materially influence the formation of functional binding regions. The light chain can also be a light chain as specified herein above, having 1-5 amino acid insertions, deletions, substitutions or a combination thereof.

Preferably, both the first antigen binding site and said second antigen binding site comprise a light chain variable region comprising a CDR1 having the sequence (RASQSISSYLN), a CDR2 having the sequence (AASSLQS), and a CDR3 having the sequence (QQSYSTPPT).

The term ‘ErbB-1’ as used herein refers to the protein that in humans is encoded by the ERBB-1 gene. Alternative names for the gene or protein include EGFR, ERBB, HER1, Erb-B2 receptor tyrosine kinase 1. Where reference is made herein to ErbB-1, the reference refers to human ErbB-1.

The term ‘ErbB-2’ as used herein refers to the protein that in humans is encoded by the ERBB-2 gene. Alternative names for the gene or protein include CD340; HER-2; HER-2/neu; MLN 19; NEU; NGL; TKR1. The ERBB-2 gene is frequently called HER2 (from human epidermal growth factor receptor 2). Where reference is made herein to ErbB-2, the reference refers to human ErbB-2. An antibody comprising an antigen-binding site that binds ErbB-2, binds human ErbB-2. The ErbB-2 antigen-binding site may, due to sequence and tertiary structure similarity between human and other mammalian orthologs, also bind such an ortholog but not necessarily so. Database accession numbers for the human ErbB-2 protein and the gene encoding it are (NP\_001005862.1, NP\_004439.2 NC\_000017.10 NT\_010783.15 NC\_018928.2). The accession numbers are primarily given to provide a further method of identification of ErbB-2 as a target, the actual sequence of the ErbB-2 protein bound the antibody may vary, for instance because of a mutation in the encoding gene such as those occurring in some cancers or the like. The ErbB-2 antigen binding site binds ErbB-2 and a variety of variants thereof, such as those expressed by some ErbB-2 positive cells or tumor cells.

The term 'ErbB-3' as used herein refers to the protein that in humans is encoded by the ERBB-3 gene. Alternative names for the gene or protein are HER3; LCCS2; MDA-BF-1; c-ErbB-3; c-erbb-3; erbb-3-S; p180-ErbB-3; p45-sErbB-3; and p85-sErbB-3. Where reference is made herein to ErbB-3, the reference refers to human ErbB-3. An antibody comprising an antigen-binding site that binds ErbB-3, binds human ErbB-3. The ErbB-3 antigen-binding site, may, due to sequence and tertiary structure similarity between human and other mammalian orthologs, also bind such an ortholog but not necessarily so. Database accession numbers for the human ErbB-3 protein and the gene encoding it are (NP\_001005915.1  
5 NP\_001973.2, NC\_000012.11 NC\_018923.2 NT\_029419.12 ). The accession numbers are primarily given to provide a further method of identification of ErbB-3 as a target, the actual sequence of the ErbB-3 protein bound by an antibody may vary, for instance because of a mutation in the encoding gene such as those occurring in some cancers or the like. The ErbB-3 antigen binding site binds ErbB-  
15 3 and a variety of variants thereof, such as those expressed by some ErbB-2 positive cells or tumor cells.

The term 'ErbB-4' as used herein refers to the protein that in humans is encoded by the ERBB-4 gene. Alternative names for the gene or protein include HER4, Erb-B2 receptor tyrosine kinase 4, and Human Epidermal Growth Factor  
20 Receptor 4. Where reference is made herein to ErbB-1, the reference refers to human ErbB-4.

The antibodies disclosed herein can reduce a ligand-induced receptor function of ErbB-3 on an ErbB-2 and ErbB-3 positive cell. In the presence of excess ErbB-2, ErbB-2/ErbB-3 heterodimers may provide a growth signal to the  
25 expressing cell in the absence of detectable ligand for the ErbB-3 chain in the heterodimer. This ErbB-3 receptor function is herein referred as a ligand-independent receptor function of ErbB-3. The ErbB-2/ErbB-3 heterodimer also provide a growth signal to the expressing cell in the presence of an ErbB-3 ligand. This ErbB-3 receptor function is herein referred to as a ligand-induced receptor  
30 function of ErbB-3.

The term "ErbB-3 ligand" as used herein refers to polypeptides which bind and activate ErbB-3. Examples of ErbB-3 ligands include, but are not limited to neuregulin 1 (NRG) and neuregulin 2, betacellulin, heparin-binding epidermal

growth factor, and epiregulin. The term includes biologically active fragments and/or variants of a naturally occurring polypeptide.

Preferably, the ligand-induced receptor function of ErbB-3 is ErbB-3 ligand-induced growth of an ErbB-2 and ErbB-3 positive cell. In a preferred embodiment  
5 said cell is an MCF-7 cell (ATCC® HTB-22™); an SKBR3 (ATCC® HTB-30™) cell; an NCI-87 (ATCC® CRL-5822™) cell; a BxPC-3-luc2 cell (Perkin Elmer 125058), a BT-474 cell (ATCC® HTB-20™) or a JIMT-1 cell (DSMZ no.: ACC 589).

As used herein the ligand-induced receptor function is reduced by at least 20%, preferably at least 30, 40, 50 60, or at least 70% in a particularly preferred  
10 embodiment the ligand-induced receptor function is reduced by 80, more preferably by 90%. The reduction is preferably determined by determining a ligand-induced receptor function in the presence of a bispecific antibody disclosed herein, and comparing it with the same function in the absence of the antibody, under otherwise identical conditions. The conditions comprise at least the presence of an  
15 ErbB-3 ligand. The amount of ligand present is preferably an amount that induces half of the maximum growth of an ErbB-2 and ErbB-3 positive cell line. The ErbB-2 and ErbB-3 positive cell line for this test is preferably the MCF-7 cell line (ATCC® HTB-22™), the SKBR3 cell line (ATCC® HTB-30™) cells, the JIMT-1 cell line (DSMZ ACC 589) or the NCI-87 cell line (ATCC® CRL-5822™). The test and/or  
20 the ligand for determining ErbB-3 ligand-induced receptor function is preferably a test for ErbB-3 ligand induced growth reduction as specified in the examples.

The ErbB-2 protein contains several domains (see for reference figure 1 of Landgraf, R Breast Cancer Res. 2007; 9(1): 202-). The extracellular domains are referred to as domains I-IV. The place of binding to the respective domains of  
25 antigen-binding sites of antibodies described herein has been mapped. A bispecific antibody with an antigen-binding site (first antigen-binding site) that binds domain I or domain IV of ErbB-2 (first antigen-binding site) comprises a heavy chain variable region that maintains significant binding specificity and affinity for ErbB-2 when combined with various light chains. Bispecific antibodies with an  
30 antigen-binding site (first antigen-binding site) that binds domain I or domain IV of ErbB-2 (first antigen-binding site) and an antigen-binding site for ErbB-3 (second antigen-binding site) are more effective in reducing a ligand-induced receptor function of ErbB-3 when compared to a bispecific antibody comprising an

antigen-binding site (first antigen-binding site) that binds to another extra-cellular domain of ErbB-2. A bispecific antibody comprising an antigen-binding site (first antigen-binding site) that binds ErbB-2, wherein said antigen-binding site binds to domain I or domain IV of ErbB-2 is preferred. Preferably said antigen-binding site binds to domain IV of ErbB-2. Preferred antibodies comprises a first antigen-binding site that binds domain I of ErbB-2 and a second antigen-binding site that binds domain III of ErbB-3.

In one preferred embodiment, said antibody comprises an antigen-binding site that binds at least one amino acid of domain I of ErbB-2 selected from the group consisting of T144, T164, R166, P172, G179, S180 and R181, and surface-exposed amino acid residues that are located within about 5 amino acid positions from T144, T164, R166, P172, G179, S180 or R181.

In one preferred embodiment, said antibody preferably comprises an antigen-binding site that binds at least one amino acid of domain III of ErbB-3 selected from the group consisting of R426 and surface-exposed amino acid residues that are located within 11.2 Å from R426 in the native ErbB-3 protein.

A bispecific antibody with an antigen-binding site (first antigen-binding site) that binds ErbB-2, and that further comprises ADCC are more effective than other ErbB-2 binding antibodies that did not have significant ADCC activity, particularly *in vivo*. A bispecific antibody which exhibits ADCC is therefore preferred. It was found that antibodies wherein said first antigen-binding site binds to domain IV of ErbB-2 had intrinsic ADCC activity. A domain I binding ErbB-2 binding antibody that has low intrinsic ADCC activity can be engineered to enhance the ADCC activity Fc regions mediate antibody function by binding to different receptors on immune effector cells such as macrophages, natural killer cells, B-cells and neutrophils. Some of these receptors, such as CD16A (FcγRIIIA) and CD32A (FcγRIIA), activate the cells to build a response against antigens. Other receptors, such as CD32B, inhibit the activation of immune cells. By engineering Fc regions (through introducing amino acid substitutions) that bind to activating receptors with greater selectivity, antibodies can be created that have greater capability to mediate cytotoxic activities desired by an anti-cancer Mab.

One technique for enhancing ADCC of an antibody is afucosylation. (See for instance Junttila, T. T., K. Parsons, et al. (2010). "Superior In vivo Efficacy of

Afucosylated Trastuzumab in the Treatment of HER2-Amplified Breast Cancer." Cancer Research 70(11): 4481-4489). Further provided is therefore a bispecific antibody as disclosed herein, which is afucosylated. Alternatively, or additionally, multiple other strategies can be used to achieve ADCC enhancement, for instance including glycoengineering (Kyowa Hakko/Biowa, GlycArt (Roche) and Eureka Therapeutics) and mutagenesis (Xencor and MacroGenics), all of which seek to improve Fc binding to low-affinity activating FcγRIIIa, and/or to reduce binding to the low affinity inhibitory FcγRIIb.

Several *in vitro* methods exist for determining the efficacy of antibodies or effector cells in eliciting ADCC. Among these are chromium-51 [Cr51] release assays, europium [Eu] release assays, and sulfur-35 [S35] release assays. Usually, a labeled target cell line expressing a certain surface-exposed antigen is incubated with antibody specific for that antigen. After washing, effector cells expressing Fc receptor CD16 are typically co-incubated with the antibody-labeled target cells. Target cell lysis is subsequently typically measured by release of intracellular label, for instance by a scintillation counter or spectrophotometry.

In preferred bispecific antibodies, the affinity of said second antigen-binding site for an ErbB-3 positive cell is equal to, or preferably higher than, the affinity of said first antigen-binding site for an ErbB-2 positive cell. The affinity (KD) of said second antigen-binding site for an ErbB-3 positive cell is preferably lower than or equal to 2.0 nM, more preferably lower than or equal to 1.5 nM, more preferably lower than or equal to 1.39 nM, more preferably lower than or equal to 0.99 nM. In one preferred embodiment, the affinity of said second antigen-binding site for ErbB-3 on SK-BR-3 cells is lower than or equal to 2.0 nM, more preferably lower than or equal to 1.5 nM, more preferably lower than or equal to 1.39 nM, preferably lower than or equal to 0.99 nM. In one embodiment, said affinity is within the range of 1.39-0.59 nM. In one preferred embodiment, the affinity of said second antigen-binding site for ErbB-3 on BT-474 cells is lower than or equal to 2.0 nM, more preferably lower than or equal to 1.5 nM, more preferably lower than or equal to 1.0 nM, more preferably lower than 0.5 nM, more preferably lower than or equal to 0.31 nM, more preferably lower than or equal to 0.23 nM. In one embodiment, said affinity is within the range of 0.31-0.15 nM. The above-mentioned affinities are preferably as measured using steady state cell affinity measurements, wherein

cells are incubated at 4°C using radioactively labeled antibody, where after cell-bound radioactivity is measured, as described in the Examples of WO 2015/130173.

The affinity (KD) of said first antigen-binding site for an ErbB-2 positive cell is preferably lower than or equal to 5.0 nM, more preferably lower than or equal to 4.5 nM, more preferably lower than or equal to 3.9 nM. In one preferred embodiment, the affinity of said first antigen-binding site for ErbB-2 on SK-BR-3 cells is lower than or equal to 5.0 nM, preferably lower than or equal to 4.5 nM, more preferably lower than or equal to 4.0 nM, more preferably lower than or equal to 3.5 nM, more preferably lower than or equal to 3.0 nM, more preferably lower than or equal to 2.3 nM. In one embodiment, said affinity is within the range of 3.0-1.6 nM. In one preferred embodiment, the affinity of said first antigen-binding site for ErbB-2 on BT-474 cells is lower than or equal to 5.0 nM, preferably lower than or equal to 4.5 nM, more preferably lower than or equal to 3.9 nM. In one embodiment, said affinity is within the range of 4.5-3.3 nM. The above-mentioned affinities are preferably as measured using steady state cell affinity measurements, wherein cells are incubated at 4°C using radioactively labeled antibody, where after cell-bound radioactivity is measured, as described in the Examples of WO 2015/130173.

Preferably, the bispecific antibodies used in the disclosed methods do not significantly affect the survival of cardiomyocytes. Cardiotoxicity is a known risk factor in ErbB-2 targeting therapies and the frequency of complications is increased when trastuzumab is used in conjunction with anthracyclines thereby inducing cardiac stress.

The bispecific antibodies disclosed herein are preferably used in humans. thus, preferred antibodies are human or humanized antibodies. Tolerance of a human to a polypeptide is governed by many different aspects. Immunity, be it T-cell mediated, B-cell mediated or other is one of the variables that are encompassed in tolerance of the human for a polypeptide. The constant region of a bispecific antibody is preferably a human constant region. The constant region may contain one or more, preferably not more than 10, preferably not more than 5 amino-acid differences with the constant region of a naturally occurring human antibody. It is preferred that the constant part is entirely derived from a naturally occurring human antibody. Various antibodies produced herein are derived from a human

antibody variable domain library. As such these variable domains are human. The unique CDR regions may be derived from humans, be synthetic or derived from another organism. The variable region is considered a human variable region when it has an amino acid sequence that is identical to an amino acid sequence of the variable region of a naturally occurring human antibody, but for the CDR region. The variable region of an ErbB-2 binding VH, an ErbB-3 binding VH, or a light chain in an antibody may contain one or more, preferably not more than 10, preferably not more than 5 amino-acid differences with the variable region of a naturally occurring human antibody, not counting possible differences in the amino acid sequence of the CDR regions. Such mutations occur also in nature in the context of somatic hypermutation.

Antibodies may be derived from various animal species, at least with regard to the heavy chain variable region. It is common practice to humanize such e.g. murine heavy chain variable regions. There are various ways in which this can be achieved among which there are CDR-grafting into a human heavy chain variable region with a 3D-structure that matches the 3-D structure of the murine heavy chain variable region; deimmunization of the murine heavy chain variable region, preferably done by removing known or suspected T- or B- cell epitopes from the murine heavy chain variable region. The removal is typically by substituting one or more of the amino acids in the epitope for another (typically conservative) amino acid, such that the sequence of the epitope is modified such that it is no longer a T- or B-cell epitope.

Such deimmunized murine heavy chain variable regions are less immunogenic in humans than the original murine heavy chain variable region. Preferably a variable region or domain is further humanized, such as for instance veneered. By using veneering techniques, exterior residues which are readily encountered by the immune system are selectively replaced with human residues to provide a hybrid molecule that comprises either a weakly immunogenic or substantially non-immunogenic veneered surface. An animal as used in the invention is preferably a mammal, more preferably a primate, most preferably a human.

A bispecific antibody disclosed herein preferably comprises a constant region of a human antibody. According to differences in their heavy chain constant

domains, antibodies are grouped into five classes, or isotypes: IgG, IgA, IgM, IgD, and IgE. These classes or isotypes comprise at least one of said heavy chains that is named with a corresponding Greek letter. Preferably the constant region comprises an IgG constant region, more preferably an IgG1 constant region, preferably a mutated IgG1 constant region. Some variation in the constant region of IgG1 occurs in nature, such as for instance the allotypes G1m1, 17 and G1m3, and/or is allowed without changing the immunological properties of the resulting antibody. Typically between about 1-10 amino acid insertions, deletions, substitutions or a combination thereof are allowed in the constant region.

- 10 Preferred bispecific antibodies as disclosed herein comprise:
- at least the CDR3 sequence, preferably at least the CDR1, CDR2 and CDR3 sequences, or at least the heavy chain variable region sequence, of an ErbB-2 specific heavy chain variable region selected from the group consisting of MF2926, MF2930, MF1849; MF2973, MF3004, MF3958, MF2971, MF3025, MF2916, MF3991, MF3031, MF2889, MF2913, MF1847, MF3001, MF3003 and MF1898, or a heavy chain variable region sequence that differs in at most 15 amino acids, preferably in at most 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, more preferably in at most 1, 2, 3, 4 or 5 amino acids, from the recited heavy chain variable region sequences; and/or
  - 20 - at least the CDR3 sequence, preferably at least the CDR1, CDR2 and CDR3 sequences, or at least the heavy chain variable region sequence, of an ErbB-3 specific heavy chain variable region selected from the group consisting of MF3178; MF3176; MF3163; MF3099; MF3307; MF6055; MF6056; MF6057; MF6058; MF6059; MF6060; MF6061; MF6062; MF6063; MF6064; MF 6065; MF6066; MF6067; MF6068; MF6069; MF6070; MF6071; MF6072; MF6073 and MF6074, or a heavy chain variable region sequence that differs in at most 15 amino acids, preferably in at most 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids, more preferably in at most 1, 2, 3, 4 or 5 amino acids, from the recited heavy chain variable region sequences.
  - 30 CDR sequences are for instance varied for optimization purposes, preferably in order to improve binding efficacy or the stability of the antibody. Optimization is for instance performed by mutagenesis procedures where after the stability and/or binding affinity of the resulting antibodies are preferably tested and an improved

ErbB-2 or ErbB-3 -specific CDR sequence is preferably selected. A skilled person is well capable of generating antibody variants comprising at least one altered CDR sequence. For instance, conservative amino acid substitution is applied. Examples of conservative amino acid substitution include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another hydrophobic residue, and the substitution of one polar residue for another polar residue, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine.

Preferred antibodies comprise a variable domain that binds ErbB-2, wherein the VH chain of said variable domain comprises the amino acid sequence of VH chain MF2926; MF2930; MF1849; MF2973; MF3004; MF3958 (is humanized MF2971); MF2971; MF3025; MF2916; MF3991 (is humanized MF3004); MF3031; MF2889; MF2913; MF1847; MF3001, MF3003 or MF1898; or comprises the amino acid sequence of VH chain MF2926; MF2930; MF1849; MF2973; MF3004; MF3958 (is humanized MF2971); MF2971; MF3025; MF2916; MF3991 (is humanized MF3004); MF3031; MF2889; MF2913; MF1847; MF3001, MF3003 or MF1898 as having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 more preferably at most 1, 2, 3, 4 or 5, amino acid insertions, deletions, substitutions or a combination thereof with respect to the above mentioned VH chain sequence. The VH chain of the variable domain that binds ErbB-2 preferably comprises the amino acid sequence of:

- MF1849; or
- MF2971 or a humanized version thereof, wherein said humanized version preferably comprises the amino acid sequence of MF3958; or
- MF3004 or a humanized version thereof, wherein said humanized version preferably comprises the amino acid sequence of MF3991. In one embodiment, the VH chain of the variable domain that binds ErbB-2 comprises the amino acid sequence of VH chain MF1849; or MF2971 or a humanized version thereof, wherein said humanized version preferably comprises the amino acid sequence of MF3958; or MF3004 or a humanized version thereof, wherein said humanized version preferably comprises the amino acid sequence of MF3991, wherein the recited VH sequences have at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably at most 1, 2, 3, 4 or 5, amino acid insertions, deletions, substitutions or a

combination thereof with respect to the respective sequence. In a preferred embodiment the VH chain of the variable domain that binds ErbB-2 comprises the amino acid sequence of MF3958; or comprises the amino acid sequence of MF3958 having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably at most 1, 2, 3, 4 or 5, amino acid insertions, deletions, substitutions or a combination thereof with respect to the VH chain sequence.

The VH chain of the variable domain that binds Erb-B3 preferably comprises the amino acid sequence of VH chain MF3178; MF3176; MF3163; MF3099; MF3307; MF6055; MF6056; MF6057; MF6058; MF6059; MF6060; MF6061; MF6062; MF6063; MF6064; MF 6065; MF6066; MF6067; MF6068; MF6069; MF6070; MF6071; MF6072; MF6073 or MF6074; or comprises the amino acid sequence of VH chain MF3178; MF3176; MF3163; MF3099; MF3307; MF6055; MF6056; MF6057; MF6058; MF6059; MF6060; MF6061; MF6062; MF6063; MF6064; MF 6065; MF6066; MF6067; MF6068; MF6069; MF6070; MF6071; MF6072; MF6073 or MF6074 having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably at most 1, 2, 3, 4 or 5, amino acid insertions, deletions, substitutions or a combination thereof with respect to the VH chain sequence. The VH chain of the variable domain that binds Erb-B3 preferably comprises the amino acid sequence of MF3178, MF3176, MF3163, MF6058, MF6061 or MF6065; or MF6061 or MF6065 having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably in at most 1, 2, 3, 4 or 5, amino acid insertions, deletions, substitutions or a combination thereof with respect to the respective VH chain sequence. In a preferred embodiment the VH chain of the variable domain that binds ErbB-3 comprises the amino acid sequence of MF3178; or comprises the amino acid sequence of MF3178 having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably at most 1, 2, 3, 4 or 5, amino acid insertions, deletions, substitutions or a combination thereof with respect to the VH chain sequence. Preferably, the above-mentioned amino acid insertions, deletions and substitutions are not present in the CDR3 region. The above-mentioned amino acid insertions, deletions and substitutions are also preferably not present in the CDR1 and CDR2 regions. The above-mentioned amino acid insertions, deletions and substitutions are also preferably not present in the FR4 region.

Preferably, the antibody comprises at least the CDR1, CDR2 and CDR3 sequences of MF1849, MF2971, MF3958, MF3004 or MF3991, most preferably at least the CDR1, CDR2 and CDR3 sequences of MF3958. Said antibody preferably comprises at least the CDR1, CDR2 and CDR3 sequences of MF3178, MF3176,  
5 MF3163, MF6058, MF6061 or MF6065, most preferably at least the CDR1, CDR2 and CDR3 sequence of MF3178.

Preferably, the ErbB-2 specific heavy chain variable region comprises the amino acid sequence of the VH chain MF3958 having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably at most 1, 2, 3, 4 or 5, amino acid insertions,  
10 deletions, substitutions or a combination thereof with respect said VH (preferably wherein said insertions, deletions, substitutions are not in CDR1, CDR2, or CDR3). Preferably, the ErbB-3 specific heavy chain variable region comprises the amino acid sequence of the VH chain MF3178 having at most 15, preferably 1, 2, 3, 4, 5,  
15 6, 7, 8, 9 or 10, more preferably at most 1, 2, 3, 4 or 5, amino acid insertions, deletions, substitutions or a combination thereof with respect said VH. The one or more amino acid insertions, deletions, substitutions or a combination thereof are preferably not in the CDR1, CDR2 and CDR3 region of the VH chain. They are also preferably not present in the FR4 region. An amino acid substitution is preferably a conservative amino acid substitution.

20 Preferably, the ErbB-2 specific heavy chain variable region comprises the amino acid sequence of the VH chain MF3991 having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably at most 1, 2, 3, 4 or 5, amino acid insertions, deletions, substitutions or a combination thereof with respect said VH (preferably wherein said insertions, deletions, substitutions are not in CDR1, CDR2, or CDR3).  
25 Preferably, the ErbB-3 specific heavy chain variable region comprises the amino acid sequence of the VH chain MF3178 having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably at most 1, 2, 3, 4 or 5, amino acid insertions, deletions, substitutions or a combination thereof with respect said VH. The one or more amino acid insertions, deletions, substitutions or a combination thereof are  
30 preferably not in the CDR1, CDR2 and CDR3 region of the VH chain. They are also preferably not present in the FR4 region. An amino acid substitution is preferably a conservative amino acid substitution.

Preferably, the first antigen-binding site of the antibody comprises at least the CDR1, CDR2 and CDR3 sequences of MF3958, or CDR1, CDR2 and CDR3 sequences that differ in at most three, preferably in at most two, preferably in at most one amino acid from the CDR1, CDR2 and CDR3 sequences of MF3958, and  
5 wherein said second antigen-binding site comprises at least the CDR1, CDR2 and CDR3 sequence of MF3178, or CDR1, CDR2 and CDR3 sequences that differ in at most three, preferably in at most two, preferably in at most one amino acid from the CDR1, CDR2 and CDR3 sequences of MF3178.

Preferably, the bispecific antibody comprises i) a first antigen binding site  
10 comprising an ErbB-2 specific heavy chain variable region comprising the CDR1, CDR2, and CDR3 sequence of MF3958 and a light chain variable region and ii) a second antigen binding site comprising an ErbB-3 specific heavy chain variable region comprising the CDR1, CDR2, and CDR3 sequence of MF3178 and a light chain variable region.

15 Preferably, the ErbB-2 specific heavy chain variable region has the MF3958 sequence and the ErbB-3 specific heavy chain variable region has the MF3178 sequence. This combination is also referred to as the PB4188 antibody. Preferably, the PB4188 antibody is afucosylated.

Preferably, the the bispecific antibody comprises the “heavy cahin for erbB-2  
20 binding” as depicted in the Sequence listing part 1D and the “heavy cahin for erbB-3 binding” as depicted in the Sequence listing part 1D.

Preferably, the antigen binding sites of the bispecific antibody comprise a germline light chain O12, preferably the rearranged germline human kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01 or a fragment or a functional derivative thereof  
25 (nomenclature according to the IMGT database worldwide web at [imgt.org](http://imgt.org)). The terms rearranged germline human kappa light chain IgV $\kappa$ 1-39\*01/IGJ $\kappa$ 1\*01, IGKV1-39/IGKJ1, huV $\kappa$ 1-39 light chain or in short huV $\kappa$ 1-39 are used. The light chain can have 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof. The mentioned 1, 2, 3, 4 or 5 amino acid substitutions are  
30 preferably conservative amino acid substitutions, the insertions, deletions, substitutions or a combination thereof are preferably not in the CDR3 region of the VL chain, preferably not in the CDR1, CDR2 or CDR3 region or FR4 region of the VL chain. Preferably, the first antigen binding site and the second antigen binding

site comprise the same light chain variable region, or rather, a common light chain. Preferably, the light chain variable region comprises a CDR1 having the sequence (RASQSISSYLN), a CDR2 having the sequence (AASSLQS), and a CDR3 having the sequence (QQSYSTPPT). Preferably, the light chain variable region comprises  
5 the common light chain sequence depicted the Sequence listing part 1C.

Various methods are available to produce bispecific antibodies and are discussed in WO 2015/130173. One method involves the expression of two different heavy chains and two different light chains in a cell and collecting antibody that is produced by the cell. Antibody produced in this way will typically contain a  
10 collection of antibodies with different combinations of heavy and light chains, some of which are the desired bispecific antibody. The bispecific antibody can subsequently be purified from the collection.

The ratio of bispecific to other antibodies that are produced by the cell can be increased in various ways. Preferably, the ratio is increased by expressing not  
15 two different light chains but two essentially identical light chains in the cell. This concept is in the art also referred to as the "common light chain" method. When the essentially identically light chains work together with the two different heavy chains allowing the formation of variable domains with different antigen-binding sites and concomitant different binding properties, the ratio of bispecific antibody  
20 to other antibody that is produced by the cell is significantly improved over the expression of two different light chains. The ratio of bispecific antibody that is produced by the cell can be further improved by stimulating the pairing of two different heavy chains with each other over the pairing of two identical heavy chains. The art describes various ways in which such heterodimerization of heavy  
25 chains can be achieved. One way is to generate 'knob into hole' bispecific antibodies. See US Patent Application 20030078385 (Arathoon et al. - Genentech). Another and preferred method is described in PCT application No. PCT/NL2013/050294 (WO 2013/157954 A1), which are incorporated herein by reference. Methods and means are disclosed for producing bispecific antibodies  
30 from a single cell, whereby means are provided that favor the formation of bispecific antibodies over the formation of monospecific antibodies.

Sequences referred to in the disclosure are presented below and in Figure 1.

**Sequences 1A (erbB-2 specific)****MF2926:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

5     1     GGCCCAGCCG GCCATGGCCC AGGTCCAGCT GCAGCAGTCT GGACCTGAGC TGGTGAAACC  
61    TGGGGCTTCA GTGATGATTT CCTGCAAGGC TTCTGGTTAC TCATTCACTG GCTACCACAT  
121  GAACTGGGTG AAGCAAAGTC CTGAAAAGAG CCTTGAGTGG ATTGGAGACA TAAATCCTAG  
181  CATTGGTACG ACTGCCCACA ACCAGATTTT CAGGGCCAAG GCCACAATGA CTGTTGACAA  
241  ATCCTCCAAC ACAGCCTACA TGCAGCTCAA GAGCCTGACA TCTGAAGACT CTGGAGTCTT  
10   301  TTACTGTGTT AGAAGAGGGG ACTGGTCCTT CGATGTCTGG GGCACAGGGA CCACGGTCAC  
361  CGTCTCCAGT

Amino acid sequence:

QVQLQQSGPELVKPGASVMISCKASGYSFTGYHMNWKQSPEKSLEWIGDINP  
SIGTTAHNQIFRAKATMTVDKSSNTAYMQLKSLTSEDSGVFYCVRRGDWSFDV  
15  WGTGTTVTVSS  
CDR1:       GYHMNWKQSPEKSLE  
CDR2:       NQIFRA  
CDR3:       RGDWSFDV

**20 MF2930:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

          1  GGCCCAGCCG GCCATGGCCG AGGTCCAGCT GCAGCAGTCT GGGGCTGAAC  
TGGTGAAGCC  
          61  TGGAGCCTCA GTGATGATGT CCTGTAAGGT TTCTGGCTAC ACCTTCACTT  
25  CCTATCCTAT  
          121  AGCGTGGATG AAGCAGGTTT ATGGAAAGAG CCTAGAGTGG ATTGGAAATT  
TTCATCCTTA  
          181  CAGTGATGAT ACTAAGTACA ATGAAAACCTT CAAGGGCAAG GCCACATTGA  
CTGTAGAAAA  
30           241  ATCCTCTAGC ACAGTCTACT TGGAGCTCAG CCGATTAACA TCTGATGACT  
CTGCTGTTTA  
          301  TTACTGTGCA AGAAGTAACC CATTATATTA CTTTGCTATG GACTACTGGG  
GTCAAGGAAC  
          361  CTCGGTCACC GTCTCCAGT

**35** Amino acid sequence:

EVQLQQSGAELVKPGASVMMSCKVSQYTFSTSYPIAWMKQVHGKSLEWIGNFH  
 PYSDDTKYNENFKGKATLTVEKSSSTVYLELSRLTSDDSAVYYCARSNPLYFFA  
 MDYWGQGTSVTVSS

CDR1: SYPIAWMKQVHGKSLE

5 CDR2: NENFKG

CDR3: SNPLYFFAMDY

**MF1849:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

10           1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGGAGTCT GGGGGAGGCC  
 TGGTCCAGCC  
               61 TGGGAGGTCC CTGAGACTCT CCTGTGCAGC CTCTGGATTC ACCTTCAGTA  
 GCTATGGCAT  
               121 GCACTGGGTC CGCCAGGCTC CAGGCAAGGG GCTGGAGTGG GTGGCAGTTA  
 15 TATCATATGA  
               181 TGGAAGTAAT AAATACTATG CAGACTCCGT GAAGGGCCGA TTCACCATCT  
 CCAGAGACAA  
               241 TTCCAAGAAC ACGCTGTATC TGCAAATGAA CAGCCTGAGA GCTGAGGACA  
 CGGCCGTGTA  
 20           301 TTA CTGTGCA AAAGGTGACT ACGGTTCTTA CTCTTCTTAC GCCTTTGATT  
 ATTGGGGCCA  
               361 AGGTACCCTG GTCACCGTCT CCA GT

Amino acid sequence:

QVQLVESGGGVVQPGRSLRLS CAASGFTFSSYGMHWVRQAPGKGLEWVAVISY  
 25 DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGDYGSYSS  
 YAFDYWGQGTSLVTVSS

CDR1: SYGMH

CDR2: VISYDGSNKYYADSVKG

CDR3: GDYGSYSSYAFDY

30

**MF2973:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

              1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GAAGCAGTCT GGGGCTGAGC  
 TGGTGAGGCC

61 TGGGGCTTCA GTGAAGTTGT CCTGCAAGGC TTCTGGCTAC ATTTTCACTG  
 GCTACTATAT  
 121 AAAGTGGTTG AGGCAGAGGC CTGGACAGGG ACTTGAATGG ATTGCAAAAA  
 TTTATCCTGG  
 5 181 AAGTGGTAAT ACTTACTACA ATGAGAAGTT CAGGGGCAAG GCCACACTGA  
 CTGCAGAAGA  
 241 ATCCTCCAGC ACTGCCTACA TGCAGCTCAG CAGCCTGACA TCTGAGGACT  
 CTGCTGTCTA  
 301 TTTCTGTGCA AGAGGGCCCC ACTATGATTA CGACGGCCCC TGGTTTGT  
 10 ACTGGGGCCA  
 361 AGGGACTCTG GTCACCGTCT CCAGT

Amino acid sequence:

QVQLKQSGAELVRPGASVKLSCKASGYIFTGYINWLRQRPGQGLEWIAKIYPG  
 SGNYYNEKFRGKATLTAEESSTAYMQLSSLTSEDSAVYFCARGPHYDYDGP  
 15 WFVYWGQGTLVTVSS  
 CDR1: GYYINWLRQRPGQGLE  
 CDR2: NEKFRG  
 CDR3: GPHYDYDGPWFVY

20 **MF3004:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GAAGCAGTCT GGGGCTGAGC  
 TGGTGAGGCC  
 61 TGGGGCTTCA GTGAAGCTGT CCTGCAAGGC TTCTGGCTAC ACTTTCACTG  
 25 GCTACTATAT  
 121 AAAGTGGGTG AAGCAGAGGC CTGGACAGGG ACTTGAGTGG ATTGCAAGGA  
 TTTATCCTGG  
 181 AAGTGGTTAT ACTTACTACA ATGAGAAGTT CAAGGGCAAG GCCACACTGA  
 CTGCAGAAGA  
 30 241 ATCCTCCAGC ACTGCCTACA TGCACCTCAG CAGCCTGACA TCTGAGGACT  
 CTGCTGTCTA  
 301 TTTCTGTGCA AGACCCCACT ATGGTTACGA CGACTGGTAC TTCGGTGTCT  
 GGGGCACAGG  
 361 CACCACGGTC ACCGTCTCCA GT

35 Amino acid sequence:

QVQLKQSGAELVRPGASVKLSCKASGYTFTGYINWVKQRPGQGLEWIARIYPG  
 SGYTYYNEKFKGKATLTAEESSSTAYMHLSSLTSEDSAVYFCARPHYGYDDWY  
 FGVWGTGTTVTVSS

CDR1: GYYINWVKQRPGQGLE

5 CDR2: NEKFKG

CDR3: PHYGYDDWYFGV

**MF2971:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

10           1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GAAGCAGTCT GGGGCTGAGC  
 TGGTGAGGCC  
               61 TGGGGCTTCA GTGAAACTGT CCTGCAAGGC TTCTGGCTAC ACTTTCAC TG  
 CCTACTATAT  
               121 AAAGTGGGTG AAGCAGAGGC CTGGACAGGG ACTTGAGTGG ATTGCAAGGA  
 15 TTTATCCTGG  
               181 AAGTGGCTAT ACTTACTACA ATGAGATTTT CAAGGGCAGG GCCACACTGA  
 CTGCAGACGA  
               241 ATCCTCCAGC ACTGCCTACA TGCAACTCAG CAGCCTGACA TCTGAGGACT  
 CTGCTGTCTA  
 20           301 TTTCTGTGCA AGACCTCCGG TCTACTATGA CTCGGCCTGG TTTGCTTACT  
 GGGGCCAAGG  
               361 GACTCTGGTC ACCGTCTCCA GT

Amino acid sequence:

QVQLKQSGAELVRPGASVKLSCKASGYTFTAYYINWVKQRPGQGLEWIARIYPG  
 25 SGYTYYNEIFKGRATLTADESSSTAYMQLSSLTSEDSAVYFCARPPVYYDSAWF  
 AYWGQGTTLVTVSS

CDR1: AYYINWVKQRPGQGLE

CDR2: NEIFKG

CDR3: PPVYYDSAWFAY

30

**MF3025:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

              1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GAAGCAGTCT GGGGCTGAGC  
 TGGTGAGGCC

61 TGGGACTTCA GTGAAGCTGT CCTGCAAGGC TTCTGGCTAC ACTTTCACTG  
GCTACTATAT

121 AAAGTGGGTG AAGCAGAGGC CTGGACAGGG ACTTGAGTGG ATTGCAAGGA  
TTTATCCTGG

5 181 AAGTGGTTAT ACTTACTACA ATGAGAAGTT CAAGGGCAAG GCCACACTGA  
CTGCAGAAGA

241 ATCCTCCAAC ACTGCCTATA TGCACCTCAG CAGCCTGACA TCTGAGGACT  
CTGCTGTCTA

10 301 TTTCTGTGCA AGGCCCCACT ATGGTTACGA CGACTGGTAC TTCGCTGTCT  
GGGGCACAGG

361 GACCACGGTC ACCGTCTCCA GT

Amino acid sequence:

QVQLKQSGAELVRPGTSVKLSCKASGYTFTGYINWVKQRPGQGLEWIARIYPG  
SGYTYINEKFKGKATLTAEESNTAYMHLSSLTSEDSAVYFCARPHYGYDDWY

15 FAVWGTGTTVTVSS

CDR1: GYINWVKQRPGQGLE

CDR2: NEKFKG

CDR3: PHYGYDDWYFAV

20 **MF2916:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCC AGGTCCAGCT GCAGCAGTCT GGGGCTGAGC  
TGGTGAGGCC

25 61 TGGGGCTTCA GTGAAGCTGT CCTGCAAGGC TTCTGGCTAC ACTTTCACTG  
GCTACTATAT

121 AAAGTGGGTG AAGCAGAGGC CTGGACAGGG ACTTGAGTGG ATTGCAAGGA  
TTTATCCTGG

181 CAGTGGTCAT ACTTCCTACA ATGAGAAGTT CAAGGGCAAG GCCACACTGA  
CTACAGAAAA

30 241 ATCCTCCAGC ACTGCCTACA TGCAGCTCAG CAGCCTGACA TCTGAGGACT  
CTGCTGTCTA

301 TTTCTGTGCA AGACCTATCT ACTTTGATTA CGCAGGGGGG TACTTCGATG  
TCTGGGGCAC

361 AGAACCTCG GTCACCGTCT CCACT

35 Amino acid sequence:

QVQLQQSGAELVRPGASVKLSCKASGYTFTGYINWVKQRPGQGLEWIARIYPG  
 SGHTSYNEKFKGKATLTTEKSSSTAYMQLSSLTSEDSAVYFCARPIYFDYAGGY  
 FDVWGTRTSVTVSS

CDR1: GYYINWVKQRPGQGLE

5 CDR2: NEKFKG

CDR3: PIYFDYAGGYFDV

**MF3958:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

10           1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGCAGTCT GCGCCGAAG  
 TGAAGAAACC  
               61 TGGCGCCAGC GTGAAGCTGA GCTGCAAGGC CAGCGGCTAC ACCTTCACCG  
 CCTACTACAT  
               121 CAACTGGGTC CGACAGGCC CAGGCCAGGG CCTGGAATGG ATCGGCAGAA  
 15 TCTACCCCGG  
               181 CTCCGGCTAC ACCAGCTACG CCCAGAAGTT CCAGGGCAGA GCCACCCTGA  
 CCGCCGACGA  
               241 GAGCACCAGC ACCGCTACA TGGAAGTGG CAGCCTGCGG AGCGAGGATA  
 CCGCCGTGTA  
 20           301 CTTCTGCGCC AGACCCCGG TGTACTACGA CAGCGCTTGG TTTGCCTACT  
 GGGGCCAGGG  
               361 CACCCTGGTC ACCGTCTCCA GT

Amino acid sequence:

QVQLVQSGAEVKKPGASVKLSCKASGYTFTAYINWVRQAPGQGLEWIGRIYPG  
 25 SGYTSYAQKFQGRATLTADESTSTAYMELSSLRSEDVAVYFCARPPVYYDSAWF  
 AYWGQGLTVTVSS

CDR1: AYYIN

CDR2: RIYPGSGYTSYAQKFQG

CDR3: PPVYYDSAWFAY

30

**MF3031:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

              1 GGCCCAGCCG GCCATGGCCC AGGTCCAGCT GCAGCAGTCT GGGGCTGAGC  
 TGGTGAGGCC

61 TGGGGCTTCA GTGAAGCTGT CCTGCAAGGC TTCTGGCTAC ACTTTCAC TG  
 CCTACTATAT  
 121 AAAGTGGGTG AAGCAGAGGC CTGGACAGGG ACTTGAGTGG ATTGCAAAGA  
 TTTATCCTGG  
 5 181 AAGTGGTTAT ACTTACTACA ATGAGAATTT CAGGGGCAAG GCCACACTGA  
 CTGCAGAAGA  
 241 ATCCTCCAGT ACTGCCTACA TACAACCTCAG CAGCCTGACA TCTGAGGACT  
 CTGCTGTCTA  
 301 TTTCTGTGCA AGAGGCGTCT ATGATTACGA CGGGGCCTGG TTTGCTTACT  
 10 GGGGCCAAGG  
 361 GACTCTGGTC ACCGTCTCCA GT

Amino acid sequence:

QVQLQQSGAELVRPGASVKLSCKASGYTFTAYYINWVKQRPGQGLEWIAKIYPG  
 SGYTYYNENFRGKATLTAEESSSTAYIQLSSLTSEDSAVYFCARGVYDYDGAWF  
 15 AYWGQGTLVTVSS  
 CDR1: AYYINWVKQRPGQGLE  
 CDR2: NENFRG  
 CDR3: GVDYDGAWFAY

20 **MF3991**: heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGCAGTCT GGCGCCGAAG  
 TGAAGAAACC  
 61 TGGCGCCAGC GTGAAGCTGA GCTGCAAGGC CAGCGGCTAC ACCTTCACCG  
 25 CCTACTACAT  
 121 CAACTGGGTC CGACAGGCC CAGGCCAGGG CCTGGAATGG ATCGGCAGAA  
 TCTACCCCGG  
 181 CTCCGGCTAC ACCAGCTACG CCCAGAAGTT CCAGGGCAGA GCCACCCTGA  
 CCGCCGACGA  
 30 241 GAGCACCAGC ACCGCCTACA TGGAAGTGG CAGCCTGCGG AGCGAGGATA  
 CCGCCGTGTA  
 301 CTTCTGCGCC AGACCCCACT ACGGCTACGA CGACTGGTAC TTCGGCGTGT  
 GGGGCCAGGG  
 361 CACCCTGGTC ACCGTCTCCA GT

35 Amino acid sequence:

QVQLVQSGAEVKKPGASVKLSCKASGYTFTAYYINWVRQAPGQGLEWIGRIYPG  
 SGYTSYAQKFQGRATLTADESTSTAYMELSSLRSEDTAVYFCARPHYGYDDWY  
 FGVWGQGTLVTVSS

CDR1: AYYIN  
 5 CDR2: RIYPGSGYTSYAQKFQG  
 CDR3: PHYGYDDWYFGV

**Sequences 1B (erbB-3 specific)**

**MF3178:** heavy chain variable region sequence of an erbB-3 binding antibody

10 Nucleic acid sequence (underlined sequence encodes end of leader peptide):  
 1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGCAGTCT GGGGCTGAGG  
 TGAAGAAGCC  
 61 TGGGGCCTCA GTGAAGGTCT CCTGCAAGGC TTCTGGATAC ACCTTCACCG  
 GCTACTATAT  
 15 121 GCACTGGGTG CGACAGGCC CTGGACAAGG GCTTGAGTGG ATGGGATGGA  
 TCAACCCTAA  
 181 CAGTGGTGGC ACAAATATG CACAGAAGTT TCAGGGCAGG GTCACGATGA  
 CCAGGGACAC  
 241 GTCCATCAGC ACAGCCTACA TGGAGCTGAG CAGGCTGAGA TCTGACGACA  
 20 CGGCTGTGTA  
 301 TTA CTGTGCA AGAGATCATG GTTCTCGTCA TTTCTGGTCT TACTGGGGCT  
 TTGATTATTG  
 361 GGGCCAAGGT ACCCTGGTCA CCGTCTCCAG T

Amino acid sequence:

25 QVQLVQSGAEVKKPGASVKVSKASGYTFTGYMHWVRQAPGQGLEWMGWI  
 NPNSSGGTNYAQKFQGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARDHGSRH  
 FWSYWGFYWGQGTLVTVSS  
 CDR1: GYYMH  
 CDR2: WINPNSGGTNYAQKFQG  
 30 CDR3: DHGSRHFWSYWGFY

**MF3176:** heavy chain variable region sequence of an erbB-3 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCG AGGTGCAGCT GTTGGAGTCT GGGGGAGGCT  
 35 TGGTACAGCC

61 TGGGGGGTCC CTGAGACTCT CCTGTGCAGC CTCTGGATTC ACCTTTAGCA  
GCTATGCCAT

121 GAGCTGGGTC CGCCAGGCTC CAGGGAAGGG GCTGGAGTGG GTCTCAGCTA  
TTAGTGGTAG

5 181 TGGTGGTAGC ACATACTACG CAGACTCCGT GAAGGGCCGG TTCACCATCT  
CCAGAGACAA

241 TTCCAAGAAC ACGCTGTATC TGCAAATGAA CAGCCTGAGA GCCGAGGACA  
CGGCTGTGTA

10 301 TTA CTGTGCA AGAGATTGGT GGTACCCGCC GTACTACTGG GGCTTTGATT  
ATTGGGGCCA

361 AGGTACCCTG GTCACCGTCT CCAGT

Amino acid sequence:

EVQLLES GGGLVQPGGSLRLS CAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS  
GGSTYYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARDWWYPPYYW  
15 GFDYWGQGTLVTVSS

CDR1: SYAMS

CDR2: AISGSGGSTYYADSVKG

CDR3: DWWYPPYYWGFYD

20 **MF3163:** heavy chain variable region sequence of an erbB-3 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGCAGTCT GGGGCTGAGG  
TGAAGAAGCC

25 61 TGGGGCCTCA GTGAAGGTCT CCTGCAAGGC TTCTGGATAC ACCTTCACCG  
GCTACTATAT

121 GCACTGGGTG CGACAGGCC CTGGACAAGG GCTTGAGTGG ATGGGATGGA  
TCAACCCTAA

181 CAGTGGTGGC ACAA CTATG CACAGAAGTT TCAGGGCAGG GTCACGATGA  
CCAGGGACAC

30 241 GTCCATCAGC ACAGCCTACA TGGAGCTGAG CAGGCTGAGA TCTGACGACA  
CGGCCGTGTA

301 TTA CTGTGCA AAAGATTCTT ACTCTCGTCA TTTCTACTCT TGGTGGGCCT  
TTGATTATTG

361 GGGCCAAGGT ACCCTGGTCA CCGTCTCCAG T

35 Amino acid sequence:

QVQLVQSGAEVKKPGASVKVSKASGYTFTGYMHVWRQAPGQGLEWMGWI  
 NPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCAKDSYSRH  
 FYSWWAFDYWGQGLTVTVSS

CDR1: GYYMH

5 CDR2: WINPNSGGTNYAQKFQG

CDR3: DSYSRHFYSWWAFDY

**MF3099:** heavy chain variable region sequence of an erbB-3 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

10 1 GGCCCAGCCG GCCATGGCCG AGGTCCAGCT GCAGCAGCCT GGGGCTGAGC  
 TGGTGAGGCC

61 TGGGACTTCA GTGAAGTTGT CCTGCAAGGC TTCTGGCTAC ACCTTCACCA  
 GCTACTGGAT

15 121 GCACTGGGTA AAGCAGAGGC CTGGACAAGG CCTTGAGTGG ATCGGAATTC  
 TTGATCCTTC

181 TGATAGTTAT ACTACCTACA ATCAAAAGTT CAAGGGCAAG GCCACATTAA  
 CAGTAGACAC

241 ATCCTCCAGC ATAGCCTACA TGCAGCTCAG CAGCCTGACA TCTGAGGACT  
 CTGCGCTCTA

20 301 TTA CTGTGCA AGAGGGGGAG ATTACGACGA GGGAGGTGCT ATGGACTACT  
 GGGGTCAAGG

361 AACCTCGGTC ACCGTCTCCA GT

Amino acid sequence:

EVQLQQPGAELVRPGTSVKLSCKASGYTFTSYWMHWVKRPGQGLEWIGILDP  
 25 SDSYTTYNQKFKGKATLTVDTSSSIAYMQLSSLTSEDSALYYCARGGDYDEGGA  
 MDYWGQGTSVTVSS

CDR1: SYWMH

CDR2: ILDPDSYTTYNQKFKG

CDR3: GGDYDEGGAMDY

30

**MF3307:** heavy chain variable region sequence of an erbB-3 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGCAGTCT GGGGCTGAGG  
 TGAAGAAGCC

61 TGGGGCCTCA GTGAAGGTCT CCTGCAAGGC TTCTGGATAC ACCTTCACCG  
 GCTACTATAT  
 121 GCACTGGGTG CGACAGGCC CTGGACAAGG GCTTGAGTGG ATGGGATGGA  
 TCAACCCTAA  
 5 181 CAGTGGTGGC ACAAACATATG CACAGAAGTT TCAGGGCAGG GTCACGATGA  
 CCAGGGACAC  
 241 GTCCATCAGC ACAGCCTACA TGGAGCTGAG CAGGCTGAGA TCTGACGACA  
 CGGCCGTGTA  
 301 TTACTGTGCA AGAGGTTCTC GTAAACGTCT GTCTAACTAC TTCAACGCCT  
 10 TTGATTATTG  
 361 GGGCCAAGGT ACCCTGGTCA CCGTCTCCAG T

Amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHVVRQAPGQGLEWMGWI  
 NPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARGSRKRL  
 15 SNYFNAFDYWGQGTLLVTVSS  
 CDR1: GYYMH  
 CDR2: WINPNSGGTNYAQKFQG  
 CDR3: GSRKRLSNYFNAFDY

## 20 Sequences 1C

Common Light Chain

The variable region of IGKV1-39A

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPKGKAPKLLIYAASSLQ  
 SGVPSRFSGSGSGTDFLTITSSSLQPEDFATYYCQQSYSTP  
 25 CDR 1: RASQSISSYLN  
 CDR 2: AASSLQS  
 CDR 3: QQSYSTPPT

IGKV1-39/jk1

30 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPKGKAPKLLIYAASSLQ  
 SGVPSRFSGSGSGTDFLTITSSSLQPEDFATYYCQQSYSTPPTFGGQGTKVEIK

Common light chain IGKV1-39/jk1 (constant region is underlined)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ  
 SGVPSRFRSGSGSGTDF<sup>h</sup>TLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKRTV  
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT  
EQDSKDYSLSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

5

IGKV1-39/jk5 common light chain variable domain

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ  
 SGVPSRFRSGSGSGTDF<sup>h</sup>TLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK

10 **Sequences 1D (erbB-2 specific)**

heavy chain for erbB-2 binding

QVQLVQSGAEVKKPGASVKLSCKASGYTFTAYYINWVRQAPGQGLEWIGRIYPG  
 SGYTSYAQKFQGRATLTADESTSTAYMELSSLRSED<sup>h</sup>TAVYFCARPPVYYDSAWF  
 AYWGQGT<sup>h</sup>LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT<sup>h</sup>VS  
 15 NSGALTSGVHTFPAVLQSSGLYSLSSVTV<sup>h</sup>PPSSSLGTQTYICNVNHKPSNTKVD  
 KRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV  
 SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE  
 YKCKVSNKALPAPIEKTISKAKGQPREPQVY<sup>h</sup>TDPPSREEMTKNQVSLTCEVKGF  
 YPSDIAVEWESNGQPENNYK<sup>h</sup>TTPPVLDSDGSFFLYSKLTVDKSRWQQGNV<sup>h</sup>FSC  
 20 SVMHEALHNHYTQKSLSLSPG

heavy chain for erbB-3 binding

QVQLVQSGAEVKKPGASVKVSKASGYTFTGYMH<sup>h</sup>WVRQAPGQGLEWMGWI  
 NPNSGGTNYAQKFQGRVTMTRDTSIS<sup>h</sup>TAYMELSR<sup>h</sup>LRSDDTAVYYCARDH<sup>h</sup>GSRH  
 25 FWSYWGFDYWGQGT<sup>h</sup>LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF  
 PEPVT<sup>h</sup>VS<sup>h</sup>WNSGALTSGVHTFPAVLQSSGLYSLSSVTV<sup>h</sup>PPSSSLGTQTYICNVNHK  
 PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV  
 TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ  
 DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY<sup>h</sup>TKPPSREEMTKNQV<sup>h</sup>S  
 30 LKCLVKGFYPSDIAVEWESNGQPENNYK<sup>h</sup>TTPPVLDSDGSFFLYSKLTVDKSRW  
 QQGNV<sup>h</sup>FSCSVMHEALHNHYTQKSLSLSPG

**Sequences 1E****HER2-specific Ab sequences****MF2889:** heavy chain variable region sequence of an erbB-2 binding antibody

5 Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCG AGGTCCAGCT GCAGCAGTCT GGAGCTGAGC TGGTAAGGCC  
61 TGGGACTTCA GTGAAGGTGT CCTGCAAGGC TTCTGGATAC GCCTTCACTA ATTATTTGAT  
121 AGAGTGGGTA AAGCAGAGGC CTGGCCAGGG CCTTGAGTGG ATTGGAGTGA TTTATCCTGA  
181 AGGTGGTGGT ACTATCTACA ATGAGAAGTT CAAGGGCAAG GCAACACTGA CTGCAGACAA  
10 241 ATCCTCCAGC ACTGCCTACA TGCAGCTCAG CGGCCTGACA TCTGAGGACT CTGCGGTCTA  
301 TTTCTGTGCA AGAGGAGACT ATGATTACAA ATATGCTATG GACTACTGGG GTCAAGGAAC  
361 CTCGGTCACC GTCTCCAGT

Amino acid sequence:

EVQLQQSGAELVRPGTSVKVSCKASGYAFTNYLIEWVKQRPGGLEWIGVIYPE

15 GGGTIYNEKFKGKATLTADKSSSTAYMQLSGLTSEDSAVYFCARGDYDYKYAM

DYWGQGTSVTVSS

CDR1: NYLIE

CDR2: VIYPEGGGTIYNEKFKG

CDR3: GDYDYKYAMDY

20

**MF2913:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCG AGGTCAAGCT GCAGCAGTCT GGACCTGAGC TGGTGAAGCC  
61 TGGCGCTTCA GTGAAGATAT CCTGCAAGGC TTCTGGTTAC TCATTCACTG ACTACAAAAT  
25 121 GGACTGGGTG AAGCAGAGCC ATGGAAAGAG CCTCGAATGG ATTGGAAATA TTAATCCTAA  
181 CAGTGGTGGT GTTATCTACA ACCAGAAGTT CAGGGGCAAG GTCACATTGA CTGTTGACAG  
241 GTCCTCCAGC GCAGCCTACA TGGAGCTCCG CAGCCTGACA TCTGAGGACA CTGCAGTCTA  
301 TTATTGTTCA AGAGGACTGT GGGATGCTAT GGACTCCTGG GGTCAAGGAA CCTCGGTAC  
361 CGTCTCCAGT

30 Amino acid sequence:

EVKLQQSGPELVKPGASVKISCKASGYSFTDYKMDWVKQSHGKSLEWIGNINP

NSGGVIYNQKFRGKVTTLTVDRSSSAAYMELRSLTSED TAVYYCSRGLWDAMDS

WGQGTSVTVSS

CDR1: DYKMDWVKQSHGKSLE

35 CDR2: NQKFRG

CDR3: GLWDAMDS

**MF1847:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

5 1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGGAGTCT GGGGGAGGCG TGGTCCAGCC  
 61 TGGGAGGTCC CTGAGACTCT CCTGTGCAGC CTCTGGATTC ACCTTCAGTA GCTATGGCAT  
 121 GCACTGGGTC CGCCAGGCTC CAGGCAAGGG GCTGGAGTGG GTGGCAGTTA TATCATATGA  
 181 TGGAAGTAAT AAATACTATG CAGACTCCGT GAAGGGCCGA TTCACCATCT CCAGAGACAA  
 241 TTCCAAGAAC ACGCTGTATC TGCAAATGAA CAGCCTGAGA GCTGAGGACA CGGCCGTGTA  
 10 301 TTA CTGTGCA AAAGTTGGT GGCATCCGCT GCTGTCTGGC TTTGATTATT GGGGCCAAGG  
 361 TACCCTGGTC ACCGTCTCCA GT

Amino acid sequence:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISY  
 DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGWWHPLLS  
 15 GFDYWGQGTLVTVSS

CDR1: SYGMH

CDR2: VISYDGSNKYYADSVKG

CDR3: GWWHPLLSGFDY

20 **MF3001:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCG AGGTCCAGCT GCAGCAGTCT GGGGCTGAAC TGGCAAAACC  
 61 TGGGGCCTCA GTGAAGCTGT CCTGCAAGAC TTCTGGCTAC AACTTTCCTA TCTACTGGAT  
 121 GCACTGGGTA AACAGAGGC CTGGACGGGG TCTGGAATGG ATTGGATAACA TTAATCCTAG  
 25 181 TACTGGTTAT ATTAAGAACA ATCAGAAGTT CAAGGACAAG GCCACCTGA CTGCAGACAA  
 241 ATCCTCCAAC ACAGCCTACA TGCAGCTGAA CAGCCTGACA TATGAGGACT CTGCAGTCTA  
 301 TTA CTGTACA AGAGAAGGGA TAACTGGGTT TACTTACTGG GGCCAAGGGA CTCTGGTCAC  
 361 CGTCTCCAGT

Amino acid sequence:

30 EVQLQQSGAELAKPGASVKLSCKTSGYNFPIYWMHWVKQRPGRGLEWIGYINP  
 STGYIKNNQKFKDKATLTADKSSNTAYMQLNSLTYEDSAVYYCTREGITGFTY  
 WGQGTLVTVSS

CDR1: IYWMHWVKQRPGRGLE

CDR2: NQKFKD

35 CDR3: EGITGFTY

**MF1898:** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGGAGTCT GGGGGAGGCG TGGTCCAGCC  
 61 TGGGAGGTCC CTGAGACTCT CCTGTGCAGC CTCTGGATTG ACCTTCAGTA GCTATGGCAT  
 5 121 GCACTGGGTC CGCCAGGCTC CAGGCAAGGG GCTGGAGTGG GTGGCAGTTA TATCATATGA  
 181 TGGAAGTAAT AAATACTATG CAGACTCCGT GAAGGGCCGA TTCACCATCT CCAGAGACAA  
 241 TTCCAAGAAC ACGCTGTATC TGCAAATGAA CAGCCTGAGA GCTGAGGACA CGGCCGTGTA  
 301 TTA CTGTGCA AAAGATGGTT TCCGTCGTAC TACTCTGTCT GGCTTTGATT ATTGGGGCCA  
 361 AGGTACCCTG GTCACCGTCT CCA GT

10 Amino acid sequence:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISY  
 DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDGFRRTTL  
 SGFDYWGQGTLVTVSS

CDR1: SYGMH

15 CDR2: VISYDGSNKYYADSVKG

CDR3: DGFRRTTLSGFDY

**MF3003** heavy chain variable region sequence of an erbB-2 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

20 1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GAAGCAGTCT GGACCTGAGC TGGTGAAGCC  
 61 TGGGGCCTCA GTGAAGATTT CCTGCAAGGC TTCTGGCGAC GCATTCAGTT ACTCCTGGAT  
 121 GAACTGGGTG AAGCAGAGGC CTGGAAAGGG TCTTGAGTGG ATTGGACGGA TTTATCCTGG  
 181 AGATGGAGAT ATTA ACTACA ATGGGAAGTT CAAGGGCAAG GCCACACTGA CTGCAGACAA  
 241 ATCCTCCAGC ACAGCCCACC TGCAACTCAA CAGCCTGACA TCTGAGGACT CTGCGGTCTA  
 25 301 CTTCTGTGCA AGAGGACAGC TCGGACTAGA GGCCTGGTTT GCTTATTGGG GCCAGGGGAC  
 361 TCTGGTCACC GTCTCCAGT

Amino acid sequence:

QVQLKQSGPELVKPGASVKISCKASGDAFSYSWMNWVKQRPGKGLEWIGRIYP  
 GDGDINYN GKFKGKATLTADKSSSTAHLQLNSLTSEDSAVYFCARGQLGLEAW

30 FAYWGQGTLVTVSS

CDR1: YSWMNWVKQRPGKGLE

CDR2: NGKFKG

CDR3: GQLGLEAWFAY

**HER3-specific Ab sequences****MF6058:** heavy chain variable region sequence of an erbB-3 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGCAGTCT GGGGCTGACG  
 5 TGAAGAAGCC  
 61 TGGGGCCTCA GTGAAGGTCA CGTGCAAGGC TTCTGGATAC ACCTTCACCG  
 GCTACTATAT  
 121 GCACTGGGTG CGACAGGCC CTGGACAAGC TCTTGAGTGG ATGGGATGGA  
 TCAACCCTCA  
 10 181 AAGTGGTGGC ACAAATATG CAAAGAAGTT TCAGGGCAGG GTCTCTATGA  
 CCAGGGAGAC  
 241 GTCCACAAGC ACAGCCTACA TGCAGCTGAG CAGGCTGAGA TCTGACGACA  
 CGGCTACGTA  
 301 TTACTGTGCA AGAGATCATG GTTCTCGTCA TTTCTGGTCT TACTGGGGCT  
 15 TTGATTATTG  
 361 GGGCCAAGGT ACCCTGGTCA CCGTCTCCAG T

Amino acid sequence:

QVQLVQSGADVKKPGASVKVTCKASGYTFTGYMHWVRQAPGQALEWMGWI  
 NPQSGGTNYAKKFQGRVSMTRETSTSTAYMQLSRLRSDDTATYYCARDHGSRH  
 20 FWSYWGFYWGQGLVTVSS  
 CDR1: GYYMH  
 CDR2: WNPQSGGTNYAKKFQG  
 CDR3: DHGSRHFWSYWGFY

**25 MF6061:** heavy chain variable region sequence of an erbB-3 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGCAGTCT GGGGCTGAGG  
 TGAAGAAGCC  
 61 TGGGGCCTCA GTGAAGGTCT CCTGCAAGGC TTCTGGATAC ACCTTCACCG  
 30 GCTACTATAT  
 121 GCACTGGGTG CGACAGGCC CTGGACAAGG GCTTGAGTGG ATGGGATGGA  
 TCAACCCTCA  
 181 GAGTGGTGGC ACAAATATG CACAGAAGTT TAAGGGCAGG GTCACGATGA  
 CCAGGGACAC  
 35 241 GTCCACCAGC ACAGCCTACA TGGAGCTGAG CAGGCTGAGA TCTGACGACA  
 CGGCTGTGTA

301 TTACTGTGCA AGAGATCATG GTTCTCGTCA TTTCTGGTCT TACTGGGGCT  
TTGATTATTG

361 GGGCCAAGGT ACCCTGGTCA CCGTCTCCAG T

Amino acid sequence:

5 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHWVRQAPGQGLEWMGWI  
NPQSGGTNYAQKFKGRVTMTRDTSTSTAYMELSRRLSDDTAVYYCARDHGSRH  
FWSYWGFYWGQGLVTVSS

CDR1: GYYMH

CDR2: WINPQSGGTNYAQKFKG

10 CDR3: DHGSRHFWSYWGFY

**MF6065:** heavy chain variable region sequence of an erbB-3 binding antibody

Nucleic acid sequence (underlined sequence encodes end of leader peptide):

1 GGCCCAGCCG GCCATGGCCC AGGTGCAGCT GGTGCAGTCT GGGGCTGAGG  
15 TGAAGAAGCC

61 TGGGGCCTCA GTGAAGGTCT CCTGCAAGGC TTCTGGATAC ACCTTCACCT  
CTTACTATAT

121 GCACTGGGTG CGACAGGCC CTGGACAAGG GCTTGAGTGG ATGGGATGGA  
TCAACCCTCA

20 181 GGGGGGTTCT ACAAACTATG CACAGAAGTT TCAGGGCAGG GTCACGATGA  
CCAGGGACAC

241 GTCCACCAGC ACAGTGTACA TGGAGCTGAG CAGGCTGAGA TCTGAGGACA  
CGGCTGTGTA

25 301 TTACTGTGCA AGAGATCATG GTTCTCGTCA TTTCTGGTCT TACTGGGGCT  
TTGATTATTG

361 GGGCCAAGGT ACCCTGGTCA CCGTCTCCAG T

Amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYMHWVRQAPGQGLEWMGWIN  
PQGGSTNYAQKFQGRVTMTRDTSTSTVYMELSRRLRSEDVAVYYCARDHGSRHF  
30 WSYWGFYWGQGLVTVSS

CDR1: SYMH

CDR2: WINPQGGSTNYAQKFQG

CDR3: DHGSRHFWSYWGFY

For the purpose of clarity and a concise description features are described herein as part of the same or separate embodiments, however, it will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

5

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 : Amino acid alignment of MF3178 variants.

10 Dots indicate the same amino acid as in MF3178 at that position. The CDR1, CDR2 and CDR3 sequences of MF3178 are in bold and underlined.

Figure 2: Increased *in vivo* tumor-targeting of bispecific over monoclonal antibody. Micro-PET imaging demonstrates that the PB4188 variant more effectively accumulates in tumors compared to the HER3 monoclonal (Fig. 2A). Gamma-counter quantification of radioactivity present in tumors confirmed that levels of PB4188 variant in the tumors were 2.5-fold higher than for the parental anti-HER3 antibody (Fig. 2B). Quantitative biodistribution for tumour uptake in the 4 mAb groups at 48 hrs. Results are expressed as a percentage of the injected dose per gram of tissue (%ID/g), error bars indicate  $\pm S$  (Fig. 2C)

15  
20

Figure 3: Antibody antagonist mode dose response curves in EGFR:HER2, HER2:HER3 and HER2:HER4 assays. Reporter cells were seeded for 4hr at 37°C at 2.5K/well in the case of EGFR:HER2 or 5K/well in the case of HER2:HER3 and HER2:HER4. Antibodies were serially diluted and incubated for 3hr at 37°C prior to stimulation for 16 hrs with 10ng/ml EGF or 30 ng/ml HRG- $\beta$ 2 in the case of EGFR:HER2 or HER2:HER3 and HER2:HER4, respectively. Reference stimulation curves of agonists were obtained by incubating titrations of ligands alone for 24 hrs. Each data point represents the mean and standard deviation of four replicates per dose. Data were plotted in GraphPad Prism and curve fits were performed using a log (inhibitor) vs response- variable response (4 parameters) fit to calculate IC50's.

25  
30

Figure 4: Body Weight Changes of Mice in the Different Groups. Body weight changes after administering MCLA-128, PG2863 and PG2869 antibodies to female BALB/c nude mice bearing OV-10-0050 established tumors. Data points represent group mean body weight. Error bars represent standard error of the mean (SEM).

5

Figure 5: Relative Change of Body Weights (%). BW change was calculated based on animal weight on the first day of dosing. Data points represent percent group mean change in BW. Error bars represent standard error of the mean (SEM).

10 Figure 6: Tumor Growth Curve. Tumor volume trace after administering MCLA-128, PG2863 and PG2869 antibodies to female BALB/c nude mice bearing OV-10-0050 established tumors. Data points represent group mean, error bars represent standard error of the mean (SEM).

15 Figure 7: Growth inhibition of tumor line MDA-MB-175 and OV-10-0050 in vitro and in vivo.

The DOC4-NRG1 and CLU-NRG1 gene fusions are expressed in the MDA-MB-175 cell line (breast) and in the OV-10-0050 PDX (ovarian), respectively. Left panel: in vitro MCLA-128 treatment inhibits MDA-MB-175 cell proliferation. Right panel: In vivo, MCLA-128 treatment (25 mg/kg weekly until day 28) reduced tumor growth and eliminated tumors in 6/8 animals.

20

## EXAMPLES

### Example 1: ErbB-2-guided targeting

25 An imaging experiment was performed comparing the HER2xHER3 bispecific antibody (PB4188) to the HER3 bivalent monoclonal antibody. Variants of bAb PB4188 and anti-HER3 MF3178 (parental antibody) were labelled with  $^{64}\text{Cu}$  and injected intravenously in mouse xenografted with HER2 gene-amplified JIMT-1 tumors. Micro-PET imaging demonstrated that the PB4188 variant more  
30 effectively accumulated in tumors compared to the HER3 monoclonal (Fig. 2A). Gamma-counter quantification of radioactivity present in tumors confirmed that levels of PB4188 variant in the tumors were 2.5-fold higher than for the parental

anti-HER3 antibody (Fig. 2B). Overall, in vitro and in vivo data demonstrate that HER2-targeting is responsible for enhanced binding of PB4188 on tumor cells.

Additional studies were performed using an anti-HER2 (MF3958) antibody. Figure 2C summarizes the results of the respective antibodies labelled with  $^{64}\text{Cu}$  and  
5 injected in mouse xenografted with HER2 gene-amplified JIMT-1 tumors (n=4 mice for each antibody treatment).

#### Methods

Biodistribution study. Variants of bAb PB4188, anti-HER2 MF3958, and anti-HER3 MF3178 were conjugated to a bifunctional chelator [Paterson 2014 Dalton

10 Transactions]. Binding characteristics of the conjugated products to the target were confirmed using flow cytometry-based assays. Proteins were then labelled with  $^{64}\text{Cu}$  and mice bearing JIMT-1 breast xenografts were administered the radiolabeled antibodies via tail vein (Figure 2A-B and “i.v.” for Figure 2C) or intraperitoneal (“i.p.” for Figure 2C). MicroPET/CT images were acquired 48 hrs  
15 post-injection, after which tumor were excised and radioactivity was measured in a gamma counter. Results were expressed as percentage injected dose per gram tissue.

#### **Example 2 Inhibition of heterodimer formation**

20 Heterodimerization assays based on the enzyme fragment complementation technology were used. The  $\beta$ -galactosidase enzyme can be artificially split into two inactive fragments, the enzyme donor and the enzyme acceptor, which combine into an active enzyme only when in close proximity. Each sequence encoding either the enzyme donor or the enzyme acceptor is linked to the extracellular and  
25 transmembrane domains of each heterodimerization partner. Both genes are then co-transfected in U2OS cells to express extracellular domains of RTK receptors linked to one domain of  $\beta$ -galactosidase (ED or EA). Upon agonistic stimulation of one RTK receptor, both RTK receptors dimerize, inducing formation of an active fully reconstituted  $\beta$ -galactosidase enzyme. Ultimately,  $\beta$ -galactosidase activity is  
30 measured by adding a substrate that upon hydrolyzation will lead to light emission.

Antibodies were tested in EGFR:HER2, HER2:HER3 and HER3:HER4 heterodimerization reporter cell lines. RTK heterodimerization assays were run

with the bispecific antibody MCLA-128 (MF3178 arm and MF3958 arm); anti-HER3 antibodies MF3178/PG3178 and PG3793/AMG-888/ patritumab; and anti-HER2 antibodies MF3958/PG3958, PG2867/trastuzumab, PG2869/pertuzumab, and Perjeta (clinical batch of pertuzumab). EGF and HRG titrations in

5 EGFR:HER2 and HER2:HER3, HER2:HER4 assays showed dose-dependent agonist responses (Figure 3). MCLA-128 showed complete inhibition of HER2:HER3 dimer formation specifically and had no effect on EGFR:HER2 or HER2:HER4 heterodimerization. In contrast, trastuzumab (PG2867) behaved as partial antagonist in EGFR:HER2 and HER2:HER3 assays.

10 MCLA-128 and PG3178 fully inhibited HRG-induced HER2:HER3 dimerization with the highest potency (Table 1).

Table 1: Summary results of EC50 of antibodies tested in RTK heterodimerization assays. EC50 were determined non-linear regressions (4 parameters) in Prism.

IC50 (nM)	EGFR:HER2	HER2:HER3	HER2:HER4
PG1337	-	-	-
PB4188	-	1.12	-
PG3187	-	1.27	-
PG3958	-	-	1.69
PG2867	0.30	4.91	0.63
PG2869	0.47	4.22	2.91
PG3793	-	3.23	-
Perjeta	0.61	6.26	5.44
Agonist	0.02	0.22	0.08

15

The potency of trastuzumab was about 4-fold lower than MCLA-128 or PG3178 in the HER2:HER3 assay. Perjeta (clinical pertuzumab) behaved as full antagonist in all three assays and gave a similar profile as PG2867 (pertuzumab). In HER2:HER4 assays, both anti-HER2 PG3958 and PG2867 (pertuzumab) showed

20 minor decreases in dimerization that appeared to be dose-dependent. Small non-specific responses in EGFR:HER2 assays were observed at high concentrations of PG1337, MCLA-128, PG3178 and PG3958.

MCLA-128 showed specific inhibition of HER2:HER3 heterodimers only. This indicates that upon binding on HER2, MCLA-128 should not sterically impair

25 interaction of HER2 with EGFR upon EGF stimulation, nor impair heterodimerization of HER2 with HER4 upon HRG stimulation.

The latter is in line with observation in the HRG-induced cell cycle-based proliferation assay of T47D cells. Assays using these cells failed to demonstrate inhibitory activity of MCLA-128 or PG3178, which was presumably attributed to the higher expression of HER4 compared to HER3. HRG is thought to preferably  
5 signal via HER2:HER4 in T47D cells instead of HER2:HER3, explaining the lack of efficacy of MCLA-128 and indicating a specificity of MCLA-128 for HRG-induced HER2:HER3 dimers and not for HRG-induced HER2:HER4 dimers.

In the current study, trastuzumab blocked EGF- and HRG-induced heterodimerization of EGFR:HER2 and HER2:HER3, respectively. Trastuzumab  
10 and pertuzumab behaved as partial and full antagonist, respectively, which is in line with the generally accepted claim that trastuzumab blocks ligand-independent activation of HER2 while pertuzumab inhibits ligand-dependent signaling. The fact that a trastuzumab inhibitory response is observed in these assays might be due to the overexpression of both targets. This might allow a more sensitive readout than  
15 traditional immunoprecipitation experiments.

Finally, while PG3793 showed a lower binding affinity than PG3178 on MCF-7, its lower potency in HER2:HER3 heterodimerization assay is less severe (2.5-fold difference in dimerization assay potency versus 30-fold difference in binding assay affinity). This discrepancy between binding affinity and antagonism potency has  
20 previously been observed in the case of MCLA-128 and PG3178. While PG3178 binds MCF-7 with a slightly better affinity than MCLA-128, MCLA-128 outperforms PG3178 in a cell cycle-based proliferation assay.

### **Example 3**

#### **25 Study Objective and Regulatory Compliance**

The objective of the research is to evaluate the in vivo anti-tumor efficacy of MCLA-128, PG2863 and PG2869 antibodies in the treatment of the subcutaneous human ovarian cancer PDX model of OV-10-0050 in BALB/c nude mice.

#### **30 Experimental Design**

The experimental design is indicate in table 2. In all groups, blood was sampled in 4 animals on day 2 (24 hr post first dose) and in the remaining 4 animals on day 6 (5 days post first dose). At the designated time point, 50-100 µl blood was collected

into sterile collection tubes (Microvette CB300Z clotting activator/serum, Sarstedt B.V. cat#16.440.100), allowing the samples to clot at room temperature for 45 minutes, centrifuging for 10 minutes at 3000 rpm and taking up the aqueous layer (about 20  $\mu$ l serum) into another 1.5 mL sterile Eppendorf for immediate storage at  
 5 -80°C. Samples are shipped on dry ice.

Table 1: Description of Experimental Design

Group	N <sup>a</sup>	Treatment	Dose mg/kg	Dose volume ml/kg <sup>b</sup>	Conc. mg/ml
1	8	Vehicle Control (DPBS)	--	10	--
2	8	MCLA-128	25	10	2.5
3	8	PG2863	25	10	2.5
4	8	PG2869	25	10	2.5

a. N: number of animals per group;

b. Dose volume: adjust dosing volume based on body weight 10 $\mu$ l/g.

10 All animals were treated on day 1, 8, 15, 22, 29 (weekly treatment for 5 weeks)  
 Route was I.P. for all groups

Tumor samples were harvested at 48 hr post last dose (day 31). Tumors were fixed in neutral buffered formalin (tissue : fixative ratio of at least 1:20) for 24 hrs, and  
 15 then converted to FFPE blocks.

Preparation of Neutral buffer formalin: Put one bag of PBS powder in a clear 5L-volumetric flask, added 4.5 L de-ionized water and stirred to disperse the powder to obtain a clear solution. Then added 500 ml formaldehyde to stir until a homogenous solution was achieved.

20 Materials

Animals : Species: *Mus musculus*; Strain: BALB/c nude; Age: 6-8 weeks; Sex: female  
 Body weight: 18-22 g; Number of animals: 32 mice plus spare

Animal supplier: Shanghai Sino-British SIPPR/BK Laboratory Animal Co., LTD.

Diet: Animals had free access to irradiation sterilized dry granule food during the  
 25 entire study period; Water: Animals had free access to sterile drinking water.

Antibody package and storage condition:

MCLA-128; cryovials, 10  $\times$  1.5 ml/vial at 2.5 mg/ml, stored at 4°C.

PG2863; cryovials, 10 ×1.5 ml/vial at 2.5 mg/ml, stored at 4°C

PG2869; cryovials, 10 ×1.5 ml/vial at 2.5 mg/ml, stored at 4°C

#### Generation of the PDX model

The human ovarian cancer PDX model of OV-10-0050 was originally established  
5 from a 48 years old female patient presenting grade 3 adenocarcinoma of the  
ovaries. A surgically resected clinical sample was implanted in nude mice (defined  
as passage 0, P0) and the following serial implantations were defined as P1, P2,  
etc.. The P6 tumor tissue was used for this study.

#### Tumor Implantation

10 Each mouse was implanted subcutaneously at the right flank with the OV-10-0050  
P6 tumor slices which cut by scissor (~30 mm<sup>3</sup>) for tumor development. The  
treatments were started on day 30 after tumor implantation when the average  
tumor size reached approximately 152 mm<sup>3</sup>. 32 tumor bearing mice were  
15 randomized into 4 groups with a stratified randomization method and each group  
consisted of 8 tumor-bearing mice. The day of randomization was noted as day 1,  
and it was day of start of treatment. The test articles were administered to the  
mice according to the predetermined regimen as shown in the experimental design  
table (Table 2).

#### Observations

20 All the procedures related to animal handling, care and the treatment in the study  
were performed according to the guidelines approved by the Institutional Animal  
Care and Use Committee (IACUC) of WuXi AppTec following the guidance of the  
Association for Assessment and Accreditation of Laboratory Animal Care  
(AAALAC). At the time of routine monitoring, the animals were daily checked for  
25 any effects of tumor growth and treatments on normal behavior such as mobility,  
food and water consumption (only by visual inspection), body weight gain/loss (body  
weights were measured twice weekly), eye/hair matting and any other abnormal  
effect as stated in the protocol. Death and observed clinical signs were recorded on  
the basis of the numbers of animals within each subset.

#### 30 Tumor Measurements

Tested was whether the tumor growth could be delayed or if mice could be  
cured. Tumor size was measured twice weekly in two dimensions using a caliper,  
and the volume was expressed in mm<sup>3</sup> using the formula:  $V = 0.5 a \times b^2$  where a

and b are the long and short diameters of the tumor, respectively. The tumor size was then used for calculations of T-C, T/C and TGI values. T-C was calculated with T as the median time (in days) required for the treatment group tumors to reach a predetermined size (e.g., 500 mm<sup>3</sup>), and C as the median time (in days) for the control group tumors to reach the same size. The T/C value (in percent) is an indication of antitumor effectiveness; T and C were the mean volumes of the treated and control groups, respectively, on a given day. TGI was calculated for each group using the formula:  $TGI (\%) = [1 - (T_i - T_0) / (V_i - V_0)] \times 100$ ; T<sub>i</sub> was the average tumor volume of a treatment group on a given day, T<sub>0</sub> was the average tumor volume of the treatment group on the first day of treatment, V<sub>i</sub> was the average tumor volume of the vehicle control group on the same day with T<sub>i</sub>, and V<sub>0</sub> was the average tumor volume of the vehicle group on the first day of treatment.

#### Statistical Analysis

Summary statistics, including mean and the standard error of the mean (SEM), are provided for the tumor volume of each group at each time point (detailed in table 3). Statistical analysis of difference in tumor volume among the groups and the analysis of drug interaction were conducted on the data obtained at the best therapeutic time point after the final dose (the 29th day after grouping).

A one-way ANOVA was performed to compare tumor volume among groups, and when a significant F -statistics ( $P < 0.001$ , a ratio of treatment variance to the error variance) was obtained, comparisons between groups were carried out with Games-Howell. All data were analyzed using SPSS 17.0.  $p < 0.05$  was considered to be statistically significant.

#### Results

##### Mortality, Morbidity, and Body Weight Gain or Loss

Animal body weight was monitored regularly as an indirect measure of toxicity. No groups lost weight as a result of test article administration (Figure 4) and no deaths or morbidity were observed. Thus, there does not appear to be obvious toxicity associated with administering MCLA-128, PG2863 and PG2869 antibodies to tumor-bearing BALB/c nude mice.

Body weight change in female BALB/c nude mice bearing OV-10-0050 xenografts dosed with MCLA-128, PG2863 and PG2869 antibodies are shown in Figure 4 and

Figure 5. Mean tumor volume over time in female BALB/c nude mice bearing OV-10-0050 xenografts dosed with MCLA-128, PG2863 and PG2869 antibodies are shown in Table 3. Figure 6 shows the tumor growth.

5 Table 3: Tumor volume over time (mm<sup>3</sup>)<sup>a</sup>

<b>Days b</b>	<b>Vehicle (DPBS)</b>	<b>MCLA-128 25 mg/kg</b>	<b>PG2863 25 mg/kg</b>	<b>PG2869 25 mg/kg</b>
1	152 ± 17	151 ± 21	152 ±	152 ± 21
5	239 ± 18	75 ± 12	87 ± 10	41 ± 8
8	332 ± 26	69 ± 15	115 ±	12 ± 1
12	434 ± 50	47 ± 11	98 ± 13	9 ± 1
15	520 ± 63	36 ± 10	108 ±	6 ± 1
19	598 ± 64	26 ± 8	92 ± 16	4 ± 1
22	718 ± 88	23 ± 8	106 ±	3 ± 1
26	911 ± 118	21 ± 9	91 ± 16	2 ± 1
29	1,161 ±	23 ± 11	108 ±	1 ± 0

a. Mean ± SEM; b. Study day

10

#### Results and discussion

In the study, the therapeutic efficacy of MCLA-128, PG2863 and PG2869 antibodies as a single agent in the treatment of the OV-10-0050 human ovarian cancer xenograft model was evaluated. The results of tumor sizes in different groups at  
 15 different time points after tumor inoculation are shown in the Table 3, Table 4, and Figure 4.

The mean tumor size of the vehicle treated control mice reached 1,161 mm<sup>3</sup> at day 29 after grouping. Treatment with the test articles MCLA-128, PG2863 and PG2869 antibodies at 25 mg/kg (QW x 5 weeks) produced significant antitumor  
 20 activity: their mean tumor sizes were 23, 108 and 1 mm<sup>3</sup>, respectively at the same time (T/C value = 1.95%, 9.28% and 0.06%; TGI value = 112.78%, 104.37% and 114.96%; p value = 0.002, 0.003 and 0.002, respectively) their tumor growth all delays more than 14 days, at the tumor size of 500 mm<sup>3</sup> compared with the vehicle group. Treatment causes partial regression or complete regression of the tumor. A  
 25 mouse was considered to have a partial regression (PR) when tumor volume was reduced 50% or greater of its day 1 volume for three consecutive measurement during the course of the study and ≥13.5 mm<sup>3</sup> for one or more of these three

measurements. And to have a complete regression(CR) when  $<13.5 \text{ mm}^3$  for three consecutive measurement during the course of the study. Tumor free survival was considered as no palpable tumor was detected at the end of the study.

Treatment with MCLA-128, PG2863 and PG-2869 resulted different ratio of PR, CR and TFS. The mouse amount in each group that shows PR, CR and TFS is shown in table 5. All the testing articles were tolerated well by the tumor-bearing animals. No body weight loss was observed in all of treatment groups.

In summary, the three test antibodies as single agent all produced significant antitumor activity against the OV-10-0050 human ovarian cancer xenograft model in this study. It was well tolerated by the tumor-bearing animals. The results indicated that the antibodies are safe and effective anticancer agents.

**Table 4:** Tumor growth inhibition calculation for injected MCLA-128; PG2863 and PG2869 antibodies in the OV-10-0050 model calculated based on tumor volume measurements at day 29 after grouping.

Treatment	Tumor Size ( $\text{mm}^3$ ) <sup>a</sup>	T/C <sup>b</sup> (%)	TGI (%)	T-C (days) At 500 $\text{mm}^3$	p value <sup>c</sup>
Vehicle (DPBS)	1,161 ± 182	-	-	0	-
MCLA-128 (25 mg/kg)	23 ± 11	1.95	112.78	>14	0.002
PG2863 (25 mg/kg)	108 ± 22	9.28	104.37	>14	0.003
PG2869 (25 mg/kg)	1 ± 0	0.06	114.96	>14	0.002

a. Mean ± SEM.

b. Tumor Growth Inhibition is calculated by dividing the group average tumor volume for the treated group by the group average tumor volume for the control group (T/C). For a test article to be considered to have anti-tumor activity, T/C must be 50% or less. TGI is calculated using the formula  $\text{TGI} (\%) = [1 - (T_i - T_0) / (V_i - V_0)] \times 100$ .

c. p value is calculated based on tumor size, compared with the vehicle group.

**Table 5:** PR, CR and TFS statistics for different treatments

<b>Treatment</b>	<b>PR</b>	<b>CR</b>	<b>TFS</b>
MCLA-128 (25 mg/kg)	3	5	0
PG2863 (25 mg/kg)	3	0	0
PG2869 (25 mg/kg)	0	8	3

**Example 4**

MCLA-128 is a bispecific antibody targeting HER2 and HER3 receptor tyrosine  
 5 kinases (RTK), which are involved in the proliferation and survival of cancer cells. MCLA-128 has been extensively studied in the context of heregulin (HRG)-induced HER3 signaling and proliferation. It has demonstrated stronger in vitro potency than: the combination of anti-HER2 antibodies pertuzumab (PG2869) +  
 trastuzumab (PG2867), which can block ligand-dependent and ligand-independent  
 10 HER2:HER3 signaling, respectively [Agus 2002; Juntilla 2009]; the anti-HER3 MM-121 (PG2863), which blocks HRG-induced HER3 activation [Schoeberl 2010].

MCLA-128 also shows anti-tumor activity in cells expressing gene fusions involving the HRG gene. The MDA-MB-175 cell line contains the DOC4-NRG1 gene fusion,  
 15 which results in a proliferative autocrine loop due to NRG1 expression. This gene fusion has until this date not been discovered in a cancer patient setting [Sanchez-Valdivieso 2002].

From a panel of breast cancer cell lines, MDA-MB-175 cells were sensitive to single agent MCLA-128, demonstrating the importance of the HER3/HRG signaling axis  
 20 in this cell line (Figure 7 left panel). The activation of the HER2 in this cell line has also been demonstrated in vivo, where a single dose of pertuzumab, but not trastuzumab, inhibited orthotopic MDA-MB-175 tumor growth. Although the relevance of DOC4-NRG1 gene fusion in breast cancer patients has been debated [Sánchez-Valdivieso 2002], other gene fusions have recently gained interest. In  
 25 particular, the CD74-NRG1 fusion has been reported by independent groups in invasive mucinous adenocarcinoma, a subpopulation of non-small cell lung cancer [Fernandez-Cuesta 2014, Duruisseaux 2016]. Several other NRG1 gene fusions have also been detected, namely VAMP2-NRG1, RBPMS-NRG1 and WRN-NRG1 in lung cancer, as well as RAB2IL1-NRG1 in ovarian cancer [Jung 2015,  
 30 Dhanasekaran, 2014]. This diversity of gene fusions may be related to the location

of NRG1 gene on chromosome 8, which is susceptible to translocations [Adelaide 2003].

OV-10-0050 was found to be HER-dependent. Treatment with afatinib (an irreversible inhibitor of EGFR and HER2 that also inhibits transphosphorylation of  
5 HER3) led to tumor growth inhibition. The anti-tumor efficacy of MCLA-128 was compared to PBS (Figure 7, right panel).

Mice: NOD-SCID, Crl:NU(NCr)-Foxn1nu and BALB/c Nude mice. Antibodies are dosed at 25 mg/kg during 4 weeks. Tumor volumes are measured by caliper twice a week.

10

**Example 5: A Phase I/II Study of MCLA-128, a full length IgG1 Bispecific Antibody Targeting HER2 and HER3, in Patients with Solid Tumors**

**Study Duration:**

15           Accrual to the dose escalation part of the study (Part 1, first patient dosed on February 3, 2015), has been completed after recruiting 28 patients. The first patient in Part 2 of the study, the dose expansion phase, was dosed on 15 January 2016 in Europe. The total duration of Part 2 is approximately 25 - 32 months; however, the actual duration is influenced by several variables, e.g., overall subject  
20 recruitment rate .

**Number of Sites:**

          Up to 13 sites are estimated to be involved during the study. Additional sites may be added to ensure there is an acceptable enrollment rate or to replace  
25 non-enrolling/withdrawn sites.

**Number of Patients:**

          Twenty-eight (28) patients were enrolled in Part 1. For Part 2, at least 20 evaluable patients, and up to approximately 40, may be enrolled in the group of  
30 advanced/ metastatic non-small cell lung cancer with invasive mucinous adenocarcinoma or documented NRG1 fusion; NSCLC).

Patients who do not complete at least two cycles of study treatment due to other reasons than disease progression, are not evaluable for efficacy and are replaced in the respective group.

This Example describes Part 2. While the example describes the  
5 administration of MCLA-128, an Erb-2, Erb-3 binding bispecific antibody, the  
example is not intended to be limiting to the use of this specific embodiment and  
applies to other bispecific antibodies disclosed herein. .

**Study Objectives:*****Part 1***

<b>Objective</b>	
<i>Primary:</i>	
<ul style="list-style-type: none"> <li>Determination of the MTD and/or MRD of MCLA-128.</li> </ul>	<ul style="list-style-type: none"> <li>Evaluation of adverse events (AEs) and dose limiting toxicities (DLT).</li> </ul>
<i>Secondary:</i>	
<ul style="list-style-type: none"> <li>To characterize the safety and tolerability of MCLA-128.</li> </ul>	<ul style="list-style-type: none"> <li>Frequency and nature of AEs/serious adverse events (SAEs).</li> </ul>
<ul style="list-style-type: none"> <li>PK profile of MCLA-128.</li> </ul>	<ul style="list-style-type: none"> <li>Assessment of PK variables, including total exposure, maximum concentration (<math>C_{max}</math>) clearance, volume of distribution (V), volume of distribution at steady state (<math>V_{ss}</math>), half-life (<math>t_{1/2}</math>), <math>AUC_{0-t}</math> (area under the concentration versus time curve from time zero to time t), <math>AUC_{0-\infty}</math> (area under the concentration versus time curve), <math>t_{max}</math> (time to reach maximum concentration).</li> </ul>
<ul style="list-style-type: none"> <li>Immunogenicity of MCLA-128.</li> </ul>	<ul style="list-style-type: none"> <li>Incidence and serum titers of anti-drug antibodies against MCLA-128.</li> </ul>
<ul style="list-style-type: none"> <li>Evaluation of anti-tumor response and CBR.</li> </ul>	<ul style="list-style-type: none"> <li>Anti-tumor activity and clinical benefit assessed by RECIST v1.1 determining objective overall response</li> </ul>

	<p>rate (ORR), duration of response (DOR), progression-free survival (PFS) and survival; CBR is defined as the proportion of patients in whom a complete response (CR) or partial response (PR) or stable disease (SD) is observed (where SD duration is a minimum of 12 weeks).</p>
<p><i>Exploratory (includes optional assessments):</i></p>	
<ul style="list-style-type: none"> <li>• Presence of biomarkers and pharmacodynamic (PD) responses to MCLA-128.</li> </ul>	<ul style="list-style-type: none"> <li>• Assessment of relevant tumor biomarkers and markers of MCLA-128 activity in archival and/or fresh tumor biopsy material and blood. The following candidate biomarkers are assessed:             <ul style="list-style-type: none"> <li>○ HER2, HER3, pHER2, pHER3 &amp; heregulin;</li> <li>○ KRAS, NRAS, PIK3CA, BRAF mutation status (metastatic colorectal cancer (mCRC) patients only);</li> <li>○ circulating tumor deoxyribonucleic acid (DNA) and mutations in genes associated with</li> </ul> </li> </ul>

	<p>HER2/HER3 signaling;</p> <ul style="list-style-type: none"> <li>○ phosphorylated molecules in the MAPK and AKT signaling pathway.</li> </ul>
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**Part 2**

<b>Objective</b>	
<i>Primary (safety):</i>	
<ul style="list-style-type: none"> <li>• To characterize the safety and tolerability of MCLA-128.</li> </ul>	<ul style="list-style-type: none"> <li>• Frequency and nature of AEs.</li> </ul>
<i>Primary (efficacy):</i>	
<ul style="list-style-type: none"> <li>• To explore the relationships between the anti-tumor activity of MCLA-128 and disease-related biomarkers</li> </ul>	<p>Overall response rate (ORR), DOR, CBR (defined as the proportion of patients in whom a CR or PR is observed, or SD of a minimum duration of 12 weeks) per RECIST 1.1 as per local investigator's assessment. The relationship between anti-tumor activity and biomarkers including expression of HER2, HER3, and heregulin are explored, and serum biomarkers such as CA-125 (ovarian, endometrial) and CA-</p>

	19-9 (gastric)
<i>Secondary:</i>	
<ul style="list-style-type: none"> <li>• PK profile of MCLA-128.</li> </ul>	<ul style="list-style-type: none"> <li>• Assessment of PK variables, including total exposure, <math>C_{max}</math>, <math>V</math>, <math>V_{ss}</math>, <math>t_{1/2}</math>, <math>AUC_{0-t}</math>, <math>AUC_{0-\infty}</math>, <math>t_{max}</math>.</li> <li>• Population PK analysis</li> </ul>
<ul style="list-style-type: none"> <li>• Immunogenicity of MCLA-128.</li> </ul>	<ul style="list-style-type: none"> <li>• Incidence and serum titers of anti-drug antibodies against MCLA-128.</li> </ul>
<ul style="list-style-type: none"> <li>• Evaluation of PFS and overall survival, duration of response</li> </ul>	
<i>Exploratory (includes optional assessments):</i>	
<ul style="list-style-type: none"> <li>• Assessment of other relevant tumor biomarkers and markers of MCLA-128 activity in preferably fresh tumor sample/biopsy material or archival and blood.</li> </ul>	<ul style="list-style-type: none"> <li>• The following candidate biomarkers are assessed if sufficient sample is available:                             <ul style="list-style-type: none"> <li>• <b>Tumor sample</b> <ul style="list-style-type: none"> <li>○ pHER2, pHER3, HER2:HER3 dimerization;</li> <li>○ Heregulin and (depending on availability) mutations in cancer genes including those associated with HER2 and HER3</li> <li>○ Phosphorylated molecules in the MAPK and AKT signaling pathway.</li> </ul> </li> </ul> </li> </ul>

	<ul style="list-style-type: none"> <li>○ Heregulin-gene fusions</li> </ul> <p><b>Blood</b></p> <ul style="list-style-type: none"> <li>○ Fcγ receptor polymorphism</li> <li>○ Circulating tumor DNA and mutation analysis in cancer genes including those associated with HER2/HER3 signaling;</li> <li>○ Circulating tumor cells and HER2 status</li> </ul>
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**Study Design:**

This is a Phase I/II, open-label, multi-center, multi-national, dose escalation, single  
 5 group assignment study to assess the safety, tolerability, PK, PD, immunogenicity  
 and anti-tumor activity of MCLA-128.

The study is designed in 2 parts:

10 **Part 1**

Accrual to Part 1 of the study was met on 24 November 2015 and as of 24 January  
 2017 all Part 1 patients had completed the study. Nine dose levels were  
 investigated: 40 mg, 80 mg, 160 mg in cohorts of 1 patient and 240 mg, 360 mg, 480  
 15 mg, 600 mg, 750 mg, and 900 mg in cohorts of 3 patients. MCLA-128 was initially  
 given over approximately 60 minutes on Day 1 of a 3-week treatment cycle. During  
 Part 1 the infusion duration was extended to 2 hours with the option of increasing  
 it up to 4 hours to mitigate infusion-related reactions (IRRs).

No dose limiting toxicities (DLTs) were experienced at any of the dose levels.  
 Three additional patients were dosed in each of the 600 mg and 750 mg cohorts in  
 20 order to have sufficient PK information.

As an MTD was not reached at the dose level of 900 mg, the Data Review Committee (DRC) for MCLA-128-CL01 decided to assign the dose level of 750 mg as the RP2D of the study, based on the cumulative safety, available PK data and PK simulations.

5 **Part 2**

Part 2 includes a further characterization of the safety and tolerability of the selected dose level of MCLA-128, as well as assessment of CBR, defined as the proportion of patients with a CR, PR or durable SD (SD for at least 12 weeks in duration), in expansion groups of selected patient populations.

10

A weekly dose regimen with a 4-week cycle is evaluated in newly recruited patients consisting of a flat dose of 400 mg weekly for the first 2 cycles, with an 800 mg loading dose for the initial administration. From cycle 3, MCLA-128 is given at a dose of 400 mg weekly for 3 weeks followed by 1 week off. Mandatory pre-  
15 medication is administered to mitigate IRRs. However, corticosteroids are only mandatory prior to the loading dose of Day 1 of Cycle 1 and should only be used for subsequent infusions as per the investigator's discretion to manage IRRs.

Safety of the weekly schedule is reviewed during a run-in period after the first 5  
20 patients treated have completed at least 2 treatment cycles. The DRC reviews all safety data with a focus on incidence of grade 3-4 toxicities, incidence and severity of IRRs, and compliance. If the DRC review concludes that toxicity is unacceptable, the Sponsor continues patient enrolment with the 3-week cycle dose regimen until a sufficient number of patients have been enrolled per cohort.

25

No within-patient dose escalation is permitted in Part 2.

Patient populations of interest to be assessed in Part 2 of the study are:

- NSCLC with documented NRG1 fusions– only open for recruitment in  
30 Asia

At least 20 and up to approximately 40 patients may be enrolled in each Group (C-F) including a minimum of 10 patients per cohort treated with the weekly

recommended dose. Previously closed cohorts may be reopened.

***Duration of Treatment***

Patients in both Part 1 and 2 of the study may remain on treatment until disease  
5 progression, death, unacceptable toxicity or discontinuation for any other reason.

**Data Review Committee (DRC):**

All dose escalation decisions in Part 1 were made by a DRC who convened to  
review all available safety data and PK data. The DRC participants included the  
10 Principal Investigators (or their representatives), the Sponsor's Medical Director,  
the study Medical Monitor, study Pharmacovigilance Physician, study Project  
Manager, study Statistician, and invited experts as required (such as clinical  
pharmacology expert).

15 In Part 2, the DRC reviews the data following completion of the safety run-in  
period for the weekly dose before expanding the weekly dose regimen in all  
subsequent patients.

**Study Assessments:**

20 The study consists of a molecular pre-screening assessment up to a 4-week (28-  
day) screening period, followed by sequential treatment cycles until treatment  
withdrawal or termination for any reason. The treatment cycle duration is 3 weeks  
(21 days) for patients treated at the initial recommended dose in Part 2, and 4  
weeks (28 days) for patients treated at the weekly recommended dose in Part 2. All  
25 patients should attend an End of Treatment visit within 1 week after treatment  
cessation and a Final Study Visit 30 days after end of treatment or discontinuation  
from study.

Patients who have not progressed or withdrawn consent on completion of the Final  
30 Study Visit are followed up every 3 months for up to 2 years (approximately) to  
check their disease progression and/or survival status until the commencement of  
their next anti-cancer treatment.

Where ongoing evaluation of safety data and available PK, PD and anti-tumor activity data during the trial suggest that alternative dosing frequencies should be evaluated, or that other patient populations should be evaluated in Part 2, these modifications are clarified in a protocol amendment prior to commencing these

5 evaluations.

***Molecular Pre-screening and Screening:***

**Molecular pre-screening is performed in local laboratories qualified to perform molecular screening for NRG1 fusions.** To initiate pre-screening, a

5 patient must meet one of the following criteria:

- Histological diagnosis of IMA and documented absence of EGFR/ALK alterations. Note: IMA patients who have not performed the pre-screening test for NRG1 fusion can enter the trial.

OR

- 10
- Pathological examination does not allow IMA diagnosis but the investigator suspects the IMA based on symptoms, imaging features (e.g. localized consolidation, multiple bilateral nodules or consolidations), non-smoker and documented absence of EGFR/ALK alterations.

15 The molecular pre-screening Informed Consent Form (ICF) must be signed by NSCLC patients identified for potential study participation before the fresh or archival tumor tissue is submitted for analysis for determination of NRG1 fusion status. Testing can be performed at any time of the natural history of the disease (e.g. at diagnosis, during the first line of therapy, at progression, etc) up to a

20 maximum of one year prior to Cycle 1 Day 1. A fresh tumor sample (formalin-fixed paraffin-embedded; FFPE) or an archival tumor sample not older than 1 year, is required for the assessment of the presence of the NRG1 fusion. The sample should be submitted to a local laboratory qualified for testing by molecular profiling (PCR, next generation sequencing [DNA or RNA] or FISH) of NRG1

25 fusion status. Patients with a positive local NRG1 fusion result are then eligible to sign the main study ICF if they are willing and able to enter the main study.

***Main Informed Consent Form***

The main study ICF must be signed by all patients prior to any screening

30 procedures or assessments being conducted. The screening assessments are performed within 4 weeks prior to Cycle 1 Day 1, with the exception of the serum pregnancy test which must be conducted within 7 days of Cycle 1 Day 1. To be considered for screening, a baseline mandatory tumor sample, preferably a block,

from fresh or archival tissue is requested. The sponsor indicates the preference for fresh tissue. Archival is acceptable and should have been taken within 2 years from screening other than for NSCLC which must be within 1 year. It should be noted that for NSCLC patients, the baseline biopsy for screening is still required  
5 even if a pre-screening biopsy sample is provided for pre-screening local testing of NRG1. Following completion of all required screening assessments and confirmation of all eligibility criteria the patient can begin dosing on Cycle 1 Day 1.

### ***Safety Assessments***

10 Concurrent illnesses are captured at baseline; AEs and concomitant therapies are monitored throughout study participation. Safety assessments include reviewing Eastern Cooperative Oncology Group (ECOG) performance status, physical examination (including height and weight), vital signs and electrocardiograms (ECG). A cardiac function test of the Left Ventricular Ejection Fraction (LVEF) is  
15 also be carried out at Screening, end of Cycle 4 (or Cycle 5 Day 1), End of Study Visit, and at any time during the study if clinically indicated. Laboratory evaluations include clinical chemistry, hematology, coagulation tests, urinalysis and pregnancy testing. Note that a cytokine panel analysis was performed up until  
20 01 August 2017.

On all MCLA-128 administration days, the patients must remain at the clinic for at least 60 minutes from the time of the end of infusion (longer where there are PK samples required) for observation and repeat vital signs prior to discharge from the clinic. Further additional safety assessments should be performed as clinically  
25 indicated and, if needed, duration of stay in clinic should be increased based on Investigator's judgment.

### ***Immunogenicity Assessment***

Serum titers of anti-MCLA-128 antibodies are measured on Day 1 at pre-dose for  
30 each of Cycles 1, 2, 3, 4 and then every fourth cycle thereafter (Cycle 8, 12, 16 etc), and at the End of Treatment Visit and the Final Study Visit with a window of -3 days prior to the MCLA-128 administration.

***Pharmacokinetics Assessment***

Part 1 and Part 2 initial recommended dose schedule: In Cycle 1, blood samples are collected for PK analysis on Day 1 at pre-dose, at end of infusion (EOI), and at 1, 2, 4, 8, 24 hours post EOI, then on Day 4 (or Day 3), Day 8 and Day 15. In Cycles 2-4, only a pre-dose and EOI blood sample is collected.

Part 2 weekly recommended dose schedule: In Cycle 1, blood samples are collected for PK analysis on Day 1 at pre-dose, EOI, 2, 4, 24 hours post EOI, then predose on Days 8 and 15, and predose and EOI on Day 22. In Cycles 2 and 3, a pre-dose and EOI blood sample is collected on Day 15. In Cycle 4 blood samples are collected pre-dose on Day 1, and pre-dose and EOI on Day 15. Every 2 cycles thereafter (Cycles 6, 8, 10 etc) a pre-dose blood sample is collected on Day 15.

***Tumor Assessment***

Tumor assessment is evaluated according to RECIST version 1.1 per local investigator. Imaging is obtained at Screening and at the end of every 2 cycles of treatment for patients receiving the 3-week cycle regimen and every 6 weeks for patients receiving the 4-week cycle regimen.

***Biomarker and Pharmacodynamics Assessments***

A range of biomarker and pharmacodynamic tests are performed on archived and/or fresh tumor sample material and/or blood (liquid biopsy), depending on availability of archived or existing tumor tissue, consent for further tumor samples, and consent for specific biomarker testing.

The following candidate biomarkers are assessed in case sufficient sample is available:

- HER2, HER3, HER2:HER3 dimerization, phosphorylated HER2 (pHER2) and HER3 (pHER3) and heregulin;
- Circulating plasma tumor DNA (ctDNA) and tumor sample DNA (depending on availability) are used to examine mutations in cancer genes including those associated with HER2 and HER3 signaling
- Phosphorylated molecules in the MAPK and AKT signaling pathway;
- Fcγ receptor polymorphism;

- Circulating tumor cells for HER2;
- Heregulin-gene fusions

No germ line DNA assessment is included (except for Fcgamma receptor  
5 polymorphism).

At baseline the patient is requested to provide a mandatory tumor sample tissue,  
preferably a block, which could be from fresh or archival tissue. The sponsor  
indicates the preference for fresh tissue. Archival is acceptable and should have  
10 been taken within 2 years from screening other than for NSCLC which must be  
within 1 year. In addition the patient is requested optionally to provide a tumor  
sample/biopsy at the end of Cycle 4 and optionally at the End of Treatment Visit.

Blood samples are also taken at these time points for the purpose of liquid biopsy  
15 testing.

#### **Eligibility Criteria:**

The study enrolls patients with NSCLC.

20

#### ***General Inclusion Criteria for Part 2***

1. Age 18 years or older;
2. At least one measurable lesion according to RECIST v1.1;
3. Performance status of ECOG 0 or 1;
- 25 4. Estimated life expectancy of at least 12 weeks;
5. Toxicities incurred as a result of previous anti-cancer therapy resolved to  $\leq$ Grade 1 (as defined by NCI CTCAE v4.03), except for alopecia, lymphopenia assessed as non-clinically significant, Grade 2 sensory neurotoxicity;
- 30 6. At least a 4-week interval between the last received radiotherapy and the first scheduled day of dosing with MCLA-128 (with the exception of up to 1x8 Gy for pain palliation);

7. Complete recovery from major surgery (stable and <Grade 2 toxicity acceptable);
8. Laboratory values at Screening:
  - a. Absolute neutrophil count  $\geq 1.5 \times 10^9/L$  without colony stimulating factor support;
  - b. Platelets  $\geq 100 \times 10^9/L$ ;
  - c. Hemoglobin  $\geq 9$  g/dL or  $\geq 2.2$  mmol/L (not transfusion dependent);
  - d. Total bilirubin <1.5 times the upper limit of normal (ULN) (unless due to Gilbert's syndrome);
  - 10 e. AST (SGOT)  $\leq 2.5 \times$  ULN; ALT (SGPT)  $\leq 2.5 \times$  ULN;  $\leq 5 \times$  ULN for patients with advanced solid tumors with liver metastases; patients with confirmed bony metastases are permitted on study with isolated elevations in ALP  $>5 \times$  ULN;
  - 15 f. Serum creatinine  $\leq 1.5 \times$  ULN or estimated glomerular filtration rate (GFR) of  $>50$  mL/min based on the Cockcroft-Gault formula;
  - g. Coagulation function (INR and aPTT  $\leq 1.5$  ULN, unless on therapeutic anticoagulants)
  - h. Urine protein  $\leq 2+$  (as measured by dipstick) or  $\leq 100$  mg/24 hours urine;
- 20 9. Able to provide at baseline a mandatory tumor biopsy sample (FFPE), preferably a block, from fresh (preferred) or archival tissue. Archival tissue must be collected within 2 years before screening, other than for NSCLC which must be within 1 year.
- 25 10. Negative pregnancy test results available as defined by urine or blood human chorionic gonadotropin (hCG) test during Screening and within 7 days of Cycle 1, Day 1 in women of childbearing potential (defined as women  $\leq 50$  years of age or history of amenorrhea for  $\leq 12$  months prior to study entry);
- 30 11. Sexually active male and female patients of childbearing potential must agree to use an effective method of birth control (e.g., barrier methods with spermicides, oral or parenteral contraceptives and/or intrauterine devices) during the entire duration of the study and for 6 months after final administration of MCLA-128. *Note that sterility in female patients*

*must be confirmed in the patients' medical records and be defined as any of the following: surgical hysterectomy with bilateral oophorectomy, bilateral tubular ligation, natural menopause with last menses >1 year ago; radiation induced oophorectomy with last menses >1 year ago; chemotherapy induced menopause with 1 year interval since last menses;*

5

12. Ability to give written, informed consent prior to any study-specific Screening procedures, with the understanding that the consent may be withdrawn by the patient at any time without prejudice;

10

13. Capable of understanding the mandated and optional protocol requirements, is willing and able to comply with the study protocol procedures and has signed the main informed consent document. For any optional biopsy sampling (tissue and/or blood) and long-term sample storage, additional consent is required;

15

14. Patient with metastatic cancer who has disease progression after having received treatment with all available therapies known to convey clinical benefit.

15. Unresectable or metastatic NSCLC meeting one of the following conditions:

20

- Biopsy-proven invasive mucinous adenocarcinoma (IMA). Note: IMA patients who have not performed the pre-screening test for NRG1 fusion can enter the trial.

OR

25

- NSCLC with documented NRG1 fusion determined at in a qualified local laboratory by molecular profiling using methods such as PCR, next generation sequencing [DNA or RNA] or FISH in patients with no known driver mutations or fusions in EGFR/ALK genes.

30

16. Documented disease progression by investigator assessment on at least one line of standard therapy in the locally advanced or metastatic setting .

**Statistical Analysis:*****Part 1 and Part 2***

Anti-tumor and clinical benefit variables are summarized descriptively for each group in Part 2. Where appropriate, variables are presented in terms of absolute  
5 and relative change from baseline. Categorical data is presented as percentages and frequency tabulations.

Where appropriate, data from those patients who receive what becomes identified as the MTD or the MRD during Part 1, and those receiving the same dose in Part  
10 2, may be combined and summarized, as well as being summarized independently.

The frequency and nature of serious and non-serious AEs is assessed in absolute and relative frequencies and coded according to MedDRA medical dictionary.

***Part 1***

Data evaluation is descriptive in nature. Patient demographics, disease characteristics and pharmacokinetic and pharmacodynamic variables are summarized at each dose level. The frequency and nature of DLTs are also summarized at each dose level.

20

***Part 2***

With N=20 per cohort in Part 2, clinically meaningful observed correlation coefficients of at least 0.38 would be distinguishable from zero with 95% confidence; lesser, non-clinically meaningful observed correlations would not be  
25 distinguishable from zero. Hence 20 subjects per cohort in Part 2 is considered sufficient to explore the relationship between the anti-tumor activity of MCLA-128 and disease related biomarkers.

In the event that signs of clinical activity are seen, additional patients up to a total of approximately 40 may be recruited. With 40 patients, true clinical response rates  
30 of, for example, 10% to 50% can be estimated with reasonable precision of approximately  $\pm 5\%$  to  $\pm 8\%$ .

PK parameters are summarized for each cohort in Part 1 and each tumor group in Part 2. Arithmetic and geometric means are provided in addition to medians, range, SD and %CV. AUC is calculated according to the trapezoid rule. Serum concentration profiles against time are plotted for each group.

5

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Claims

1. Use of a bispecific antibody that comprises two heavy chain variable regions (VH),  
each of which is paired with the same light chain variable region (VL), comprising a first  
5 antigen-binding site that can bind domain I of ErbB-2 and a second antigen-binding site  
that can bind domain III of ErbB-3 in the manufacture of a medicament for the  
treatment of an individual that has an ErbB-2 and ErbB-3 positive cancer cell, wherein  
the individual has been screened to identify the ErbB-2 and ErbB-3 positive cancer cell  
to comprise an NRG1 fusion gene comprising at least a portion of the NRG1-gene fused  
10 to a sequence from a different chromosomal location.

2. A method of treating an individual that has an ErbB-2 and ErbB-3 positive cancer  
cell, comprising administering a bispecific antibody that comprises two heavy chain  
variable regions (VH), each of which is paired with the same light chain variable region  
15 (VL), comprising a first antigen-binding site that can bind domain I of ErbB-2 and a  
second antigen-binding site that can bind domain III of ErbB-3, wherein the individual  
has been screened to identify the ErbB-2 and ErbB-3 positive cancer cell to comprise an  
NRG1 fusion gene comprising at least a portion of the NRG1-gene fused to a sequence  
from a different chromosomal location.

20 3. The use of claim 1, or the method of claim 2, wherein the NRG1 fusion gene comprises  
at least the 3' end of the NRG1-gene fused to a 5' sequence from a different chromosomal  
location.

25 4. The use of claim 1 or 3, or the method of claim 2 or 3, wherein the cancer cell is  
associated with an NRG1-fusion.

5. The use or method of claim 4, wherein the cancer cell is driven by NRG1-fusion.

30 6. The use or method of any one of the preceding claims, wherein the cell is a breast  
cancer cell, an ovarian cancer cell, a lung cancer cell, such as non-small cell lung cancer,  
or a metastasis thereof.

35 7. Use of a bispecific antibody that comprises two heavy chain variable regions (VH),  
each of which is paired with the same light chain variable region (VL), comprising a first

antigen-binding site that can bind domain I of ErbB-2 and a second antigen-binding site that can bind domain III of ErbB-3 in the manufacture of a medicament for the treatment of an individual that has an ErbB-2 and ErbB-3 positive tumor or is at risk of having said tumor, wherein the individual has been screened to identify a cell of the

5 ErbB-2 and ErbB-3 positive tumor to express an NRG1 fusion gene comprising at least the 3' end of the NRG1-gene fused to a 5' sequence from a different chromosomal location.

8. A method of treating an individual that has an ErbB-2 and ErbB-3 positive tumor or

10 is at risk of having said tumor, comprising administering a bispecific antibody that comprises two heavy chain variable regions (VH), each of which is paired with the same light chain variable region (VL), comprising a first antigen-binding site that can bind domain I of ErbB-2 and a second antigen-binding site that can bind domain III of ErbB-3, wherein the individual has been screened to identify a cell of the ErbB-2 and ErbB-3

15 positive tumor to express an NRG1 fusion gene comprising at least the 3' end of the NRG1-gene fused to a 5' sequence from a different chromosomal location.

9. The use of claim 7, or the method of claim 8, wherein the tumor is breast cancer, an ovarian cancer, a lung cancer, such as non-small cell lung cancer, or a metastasis

20 thereof.

10. The use or method of any one of the preceding claims, wherein the NRG1-fusion gene expresses a protein that comprises an NRG1 EGF-like domain.

11. The use or method of any one of the preceding claims, wherein the NRG-fusion is a

25 fusion of NRG1 and a gene on human chromosome 8.

12. The use or method of claim 11, wherein the gene on human chromosome 8 encodes an excreted protein or a cellular membrane associated protein.

30

13. The use or method of any one of the preceding claims, wherein the NRG1 fusion gene is a fusion of the 3' end of the NRG1-gene with the 5' sequence of one of the genes selected from the group consisting of CD74; DOC4; TNFRSF10B; CLU; VAMP2; SLC3A2; RBPMS; WRN; SDC4; KIF13B; SLECA2; PDE7A; ATP1B1; CDK1; BMPR1B;

35 MCPH1 and RAB2IL1.

14. The use or method of any one of the preceding claims, wherein the cell or tumor is of an epithelial origin.

5 15. The use or method of any one of the preceding claims, wherein the individual has undergone a therapy that is targeted towards EGFR inhibition.

16. The use or method of claim 15, wherein the individual has undergone a therapy with cetuximab.

10

17. The use or method of any one of the preceding claims, wherein the ErbB-1 cell-surface receptor density; ErbB-2 cell-surface receptor density; ErbB-3 cell-surface receptor density; ErbB-4 cell-surface receptor density or a combination thereof on the cell or cells of the tumor is determined.

15

18. The use or method of any one of the preceding claims, characterized in that the cell or tumor has less than 400,000 ErbB-1 cell-surface receptors per cell.

19. The use or method of any one of the preceding claims, further comprising  
20 administering to the individual an ErbB-1 inhibitor.

20. The use or method of claim 19, wherein the ErbB-1 inhibitor is cetuximab.

21. The use or method of any one of the preceding claims, wherein the affinity of the first  
25 antigen-binding site for ErbB-2 is lower than the affinity of the second antigen-binding site for ErbB-3.

22. The use or method according to claim 21, wherein said antibody comprises

30 i) at least the CDR1, CDR2 and CDR3 sequences of an ErbB 2 specific heavy chain variable region selected from the group consisting of MF2926, MF2930, MF1849; MF2973, MF3004, MF3958, MF2971, MF3025, MF2916, MF3991, MF3031, MF2889, MF2913, MF1847, MF3001, MF3003 and MF1898; and

35 ii) at least the CDR1, CDR2 and CDR3 sequences of an ErbB 3 specific heavy chain variable region selected from the group consisting of MF3178; MF3176;

MF3163; MF3099; MF3307; MF6055; MF6056; MF6057; MF6058; MF6059;  
MF6060; MF6061; MF6062; MF6063; MF6064; MF 6065; MF6066; MF6067;  
MF6068; MF6069; MF6070; MF6071; MF6072; MF6073 and MF6074, and

5 at least a light chain variable region comprising a CDR1 sequence comprising  
RASQSISSYLN, a CDR2 sequence comprising AASSLQS, and a CDR3 sequence  
comprising QQSYSTPPT.

23. The use or method according to claim 22, wherein said antibody comprises

10 i) an ErbB 2 specific heavy chain variable region sequence selected from the  
group consisting of the heavy chain variable region sequences of MF2926,  
MF2930, MF1849, MF2973, MF3004, MF3958, MF2971, MF3025, MF2916,  
MF3991, MF3031, MF2889, MF2913, MF1847, MF3001, MF3003, and MF1898;  
and

15

ii) an ErbB 3 specific heavy chain variable region sequence selected from the  
group consisting of the heavy chain variable region sequences of MF3178;  
MF3176; MF3163; MF3099; MF3307; MF6055; MF6056; MF6057; MF6058;  
MF6059; MF6060; MF6061; MF6062; MF6063; MF6064; MF 6065; MF6066;  
20 MF6067; MF6068; MF6069; MF6070; MF6071; MF6072; MF6073 and MF6074,  
and

20

at least a light chain variable region comprising a CDR1 sequence comprising  
RASQSISSYLN, a CDR2 sequence comprising AASSLQS, and a CDR3 sequence  
25 comprising QQSYSTPPT.

25

24. The use or method of claim 22 or 23, wherein the antibody comprises at least the  
CDR1, CDR2 and CDR3 sequences of the ErbB 2 specific heavy chain variable region  
MF3958 and at least a light chain variable region comprising a CDR1 sequence  
30 comprising RASQSISSYLN, a CDR2 sequence comprising AASSLQS, and a CDR3  
sequence comprising QQSYSTPPT; and the antibody comprises at least the CDR1, CDR2  
and CDR3 sequences of the ErbB 3 specific heavy chain variable region MF3178 and at  
least a light chain variable region comprising a CDR1 sequence comprising  
RASQSISSYLN, a CDR2 sequence comprising AASSLQS, and a CDR3 sequence  
35 comprising QQSYSTPPT.

35

25. The use or method of any one of claims 22 to 24, wherein the variable domain that comprises said first antigen binding site and the variable domain that comprises said second antigen binding site comprise the same light chain variable region comprising a human germline sequence having at least a CDR1 sequence comprising RASQSISSYLN, a CDR2 sequence comprising AASSLQS, and a CDR3 sequence comprising QQSYSTPPT.

26. The use or method of claim 25, wherein the variable domain that comprises said first antigen binding site and the variable domain that comprises said second antigen binding site comprise a germline light chain O12 light chain variable region.

27. The use or method of claim 25 or 26, wherein the variable domain that comprises said first antigen binding site and the variable domain that comprises said second antigen binding site comprise a common light chain variable region comprising a rearranged human kappa light chain IgVKI-39\*01/IGJKI\*01.

Figure 1

	CDR1				CDR2			
	1	10	20	30	40	50	60	
MF3178	QVQLVQSGAEVKKPGASVKV	SCKASGYTFT	<u>GYMH</u>	WVRQAPGQGLEWMG	<u>WINPNSGGTNYAQKFOG</u>			
MF6055	.....D.....			.....A.....	.....S.....	.....K.....		
MF6056	.....D.....	T.....		.....A.....	.....S.....	.....K.....		
MF6057	.....D.....	T.....			.....Q.....			
MF6058	.....D.....	T.....		.....A.....	.....Q.....	.....K.....		
MF6059	.....				.....G.....	.....S.....		
MF6060	.....D.....			.....A.....	.....Q.....	.....K.....		
MF6061	.....				.....Q.....	.....K.....		
MF6062	.....				.....G.....	.....S.....		
MF6063	.....				.....Q.....	.....K.....		
MF6064	.....				.....K.....	.....Q.....		
MF6065	.....			S.....		.....QG.....	.....S.....	
MF6066	.....					.....Q.....	.....S.....	
MF6067	.....					.....Q.....		
MF6068	.....					.....Q.....		
MF6069	.....					.....Q.....		
MF6070	.....			S.....		.....SG.....	.....S.....	
MF6071	.....					.....S.....	.....S.....	
MF6072	.....					.....S.....		
MF6073	.....					.....S.....		
MF6074	.....					.....S.....		

	CDR3					
	70	80	90	100	110	120
MF3178	RVTMTRDTSISTAYMEL	SRLRSDDTAVYYCAR	<u>DHGSRHFWSYWGFDY</u>	WGQGTLVTVSS		
MF6055	.....E..T.....		.....T.....			
MF6056	..S..E..T.....	Q.....	.....T.....			
MF6057	.....	Q.....				
MF6058	..S..E..T.....	Q.....	.....T.....			
MF6059	.....					
MF6060	.....E..T.....		.....T.....			
MF6061	.....T.....					
MF6062	.....T.....					
MF6063	.....T.....					
MF6064	.....T.....					
MF6065	.....T..V.....		.....E.....			
MF6066	.....T.....	S.....	.....E.....			
MF6067	.....T..V.....	S.....				
MF6068	.....T.....					
MF6069	.....					
MF6070	.....T..V.....		.....E.....			
MF6071	.....T.....	S.....	.....E.....			
MF6072	.....T..V.....	S.....				
MF6073	.....T.....					
MF6074	.....					

Fig. 2

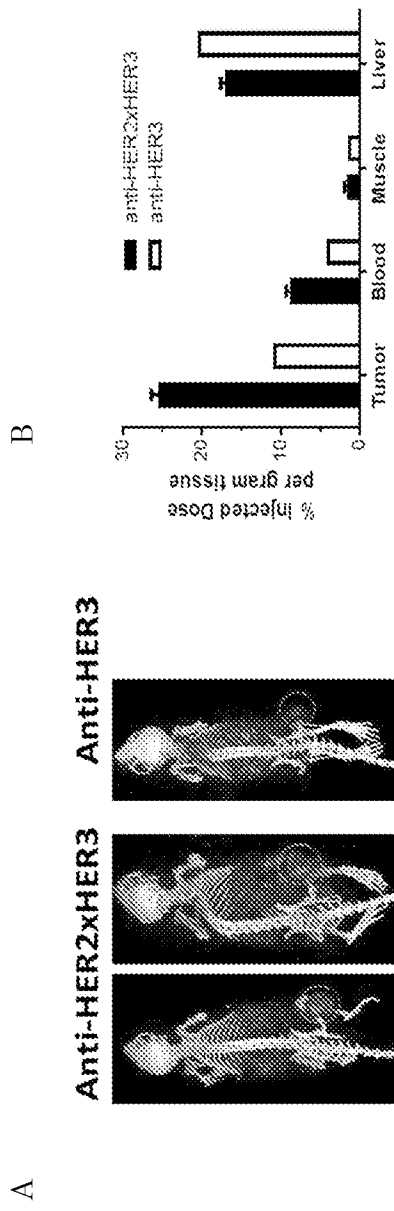
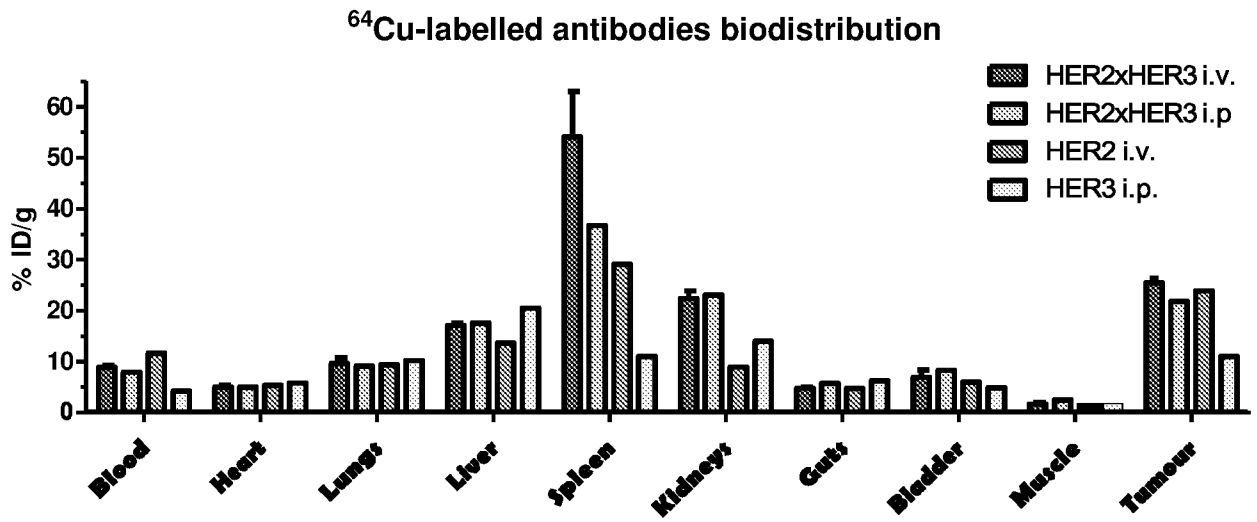


Fig 2. continued

C.



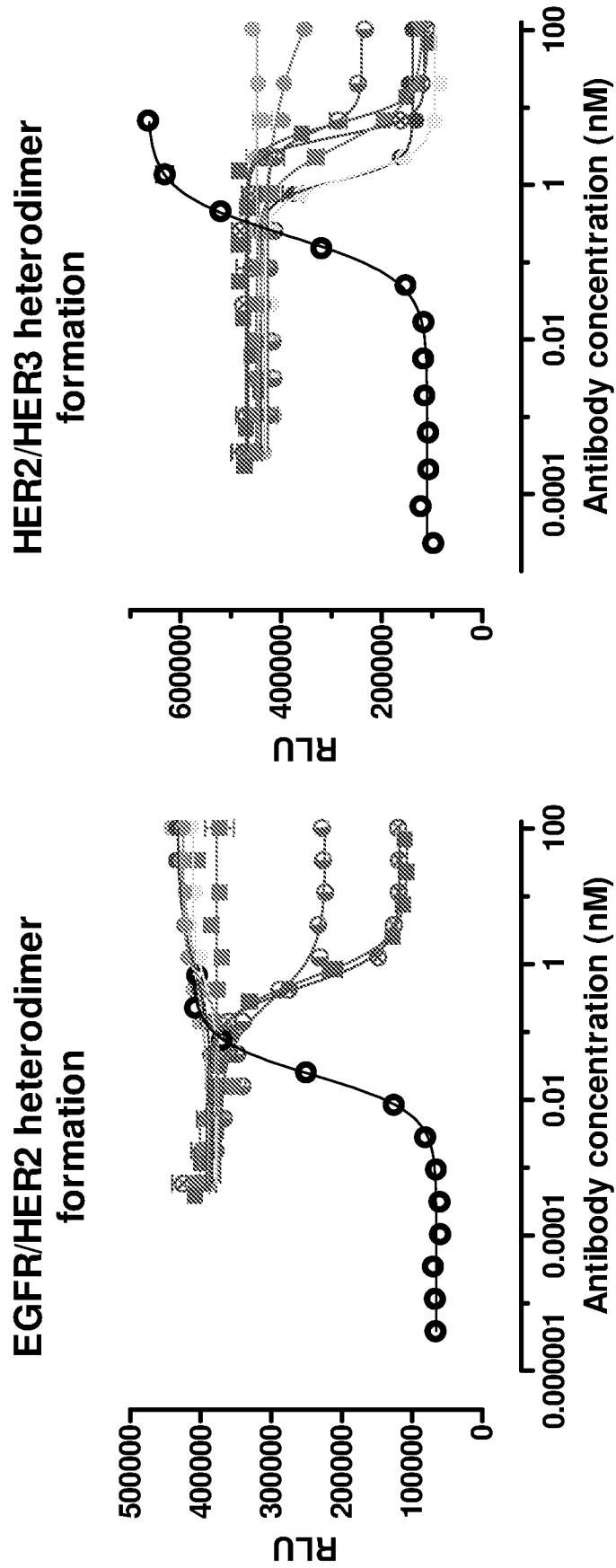


Figure 3

A

### HER2/HER4 heterodimer formation

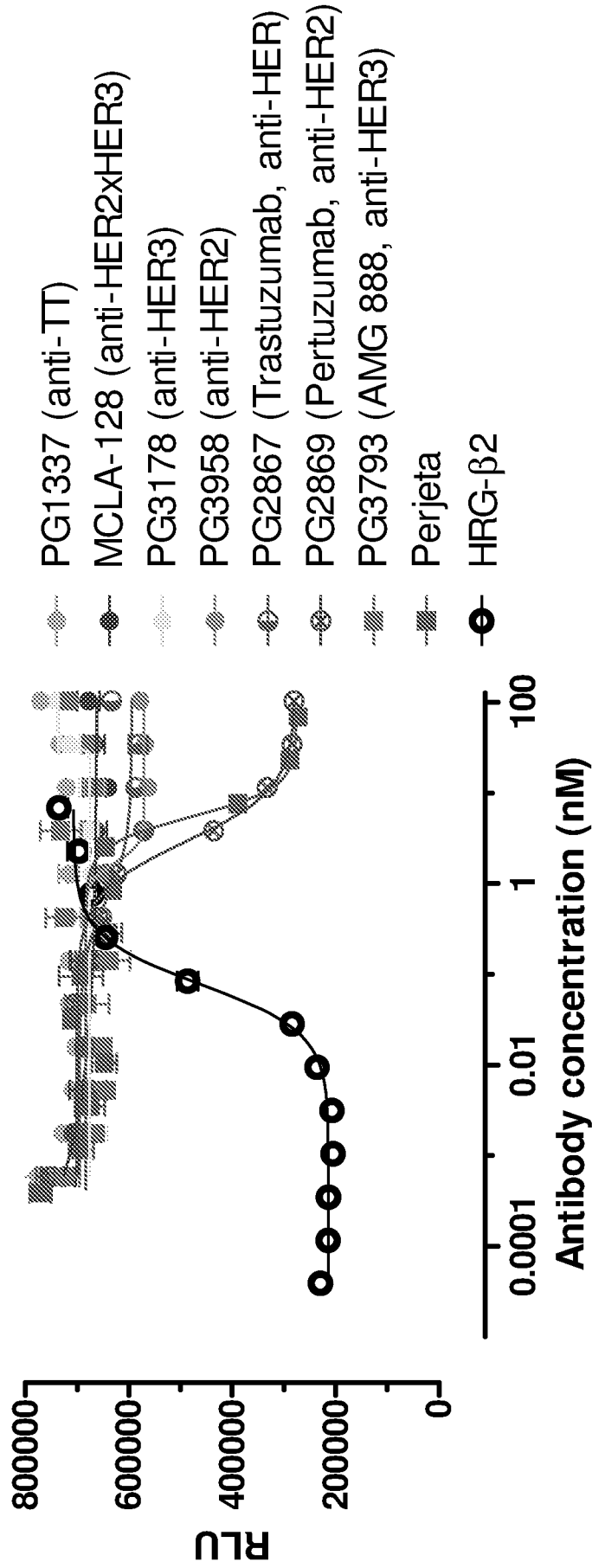
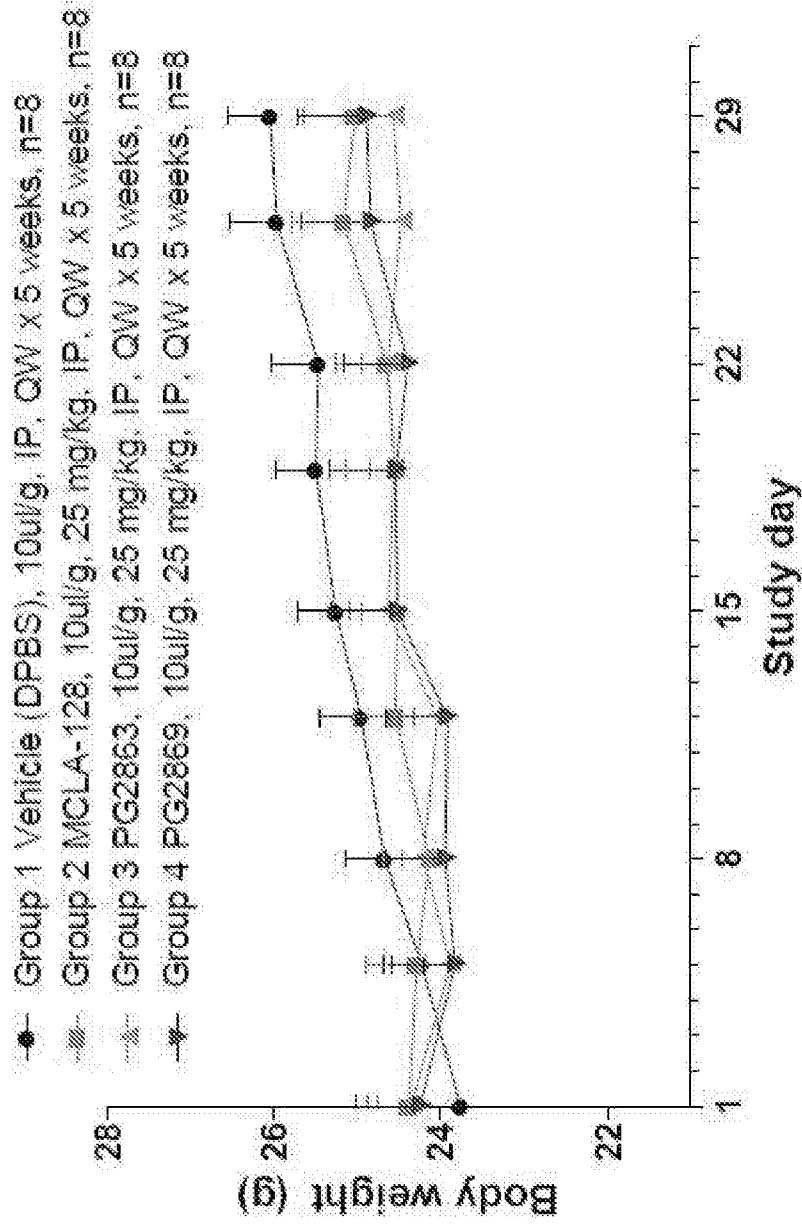


Figure 3

B

Figure 4



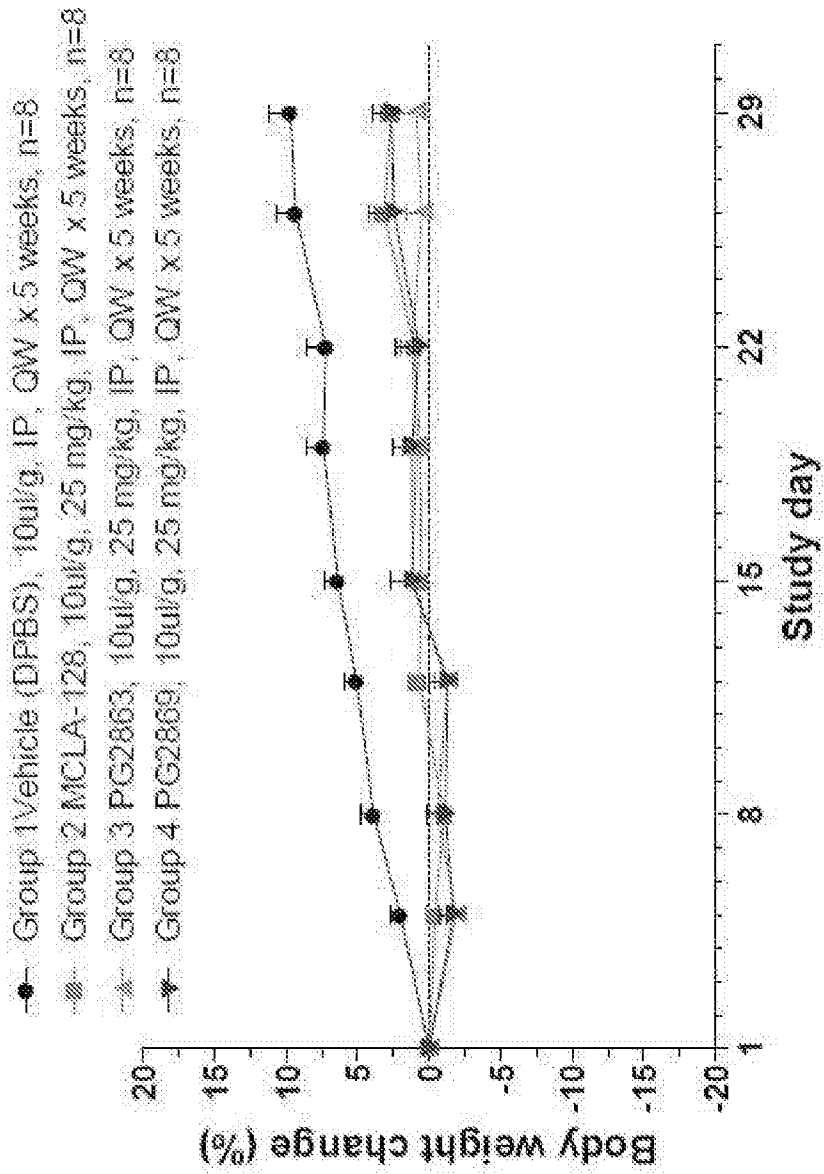


Figure 5

Figure 6

- Group 1 Vehicle (DPBS), 10ul/g, IP, QW x 5 weeks, n=8
- ▨ Group 2 MCLA-128, 10ul/g, 25 mg/kg, IP, QW x 5 weeks, n=8
- ▲ Group 3 PG2863, 10ul/g, 25 mg/kg, IP, QW x 5 weeks, n=8
- ▼ Group 4 PG2869, 10ul/g, 25 mg/kg, IP, QW x 5 weeks, n=8

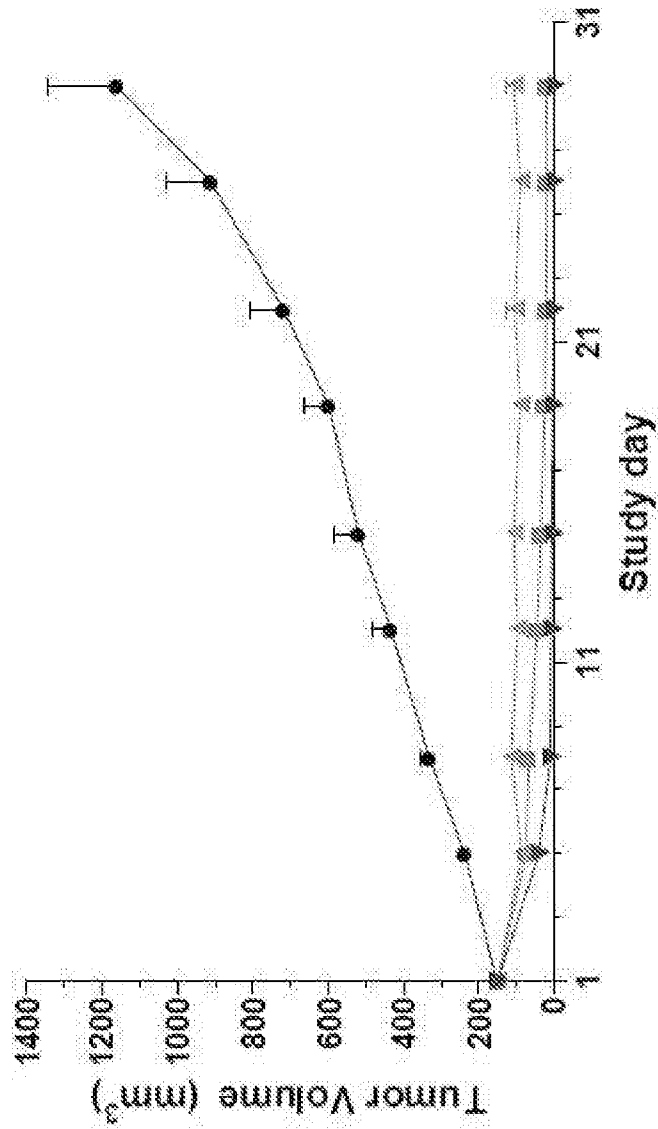


Figure 7

