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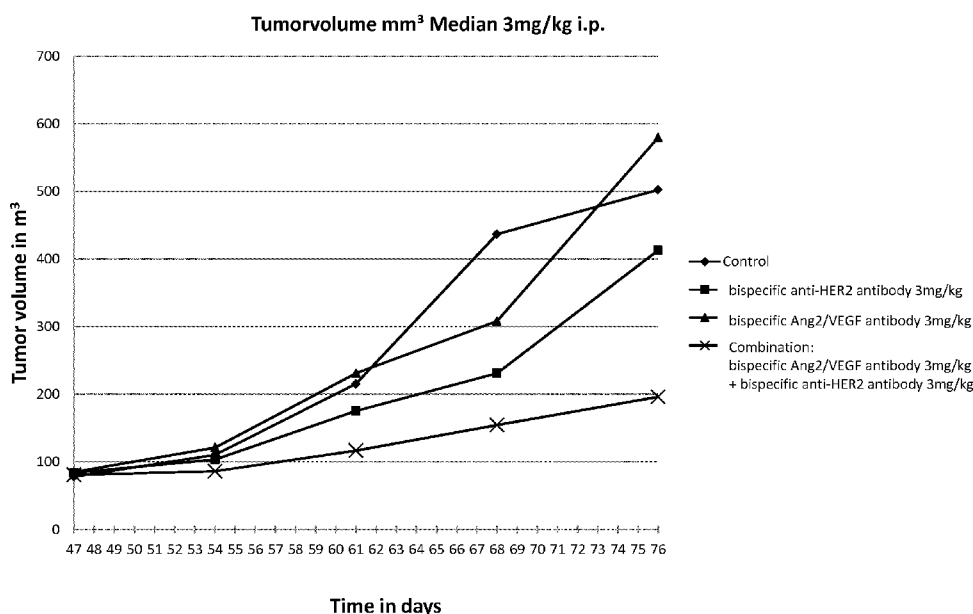
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(54) Title: COMBINATION THERAPY WITH A BISPECIFIC ANTI-ANG2/VEGF ANTIBODY AND A BISPECIFIC ANTI-HER2 ANTIBODY

Figure 1



(57) Abstract: The present invention relates to the combination of bispecific antibodies.

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Combination therapy with a bispecific anti-ANG2/VEGF antibody and a bispecific anti-HER2 antibody

The present invention relates to the combination therapy of an antibody a bispecific anti-ANG2/VEGF antibody and a bispecific anti-HER2 antibody that bind to domain II and domain IV of HER2.

Background of the Invention

5 Angiopoietins, which play a key role in angiogenesis and blood vessel remodeling, are part of the pro-angiogenic armamentarium of growing tumors. Importantly they are one of the major factors leading to secondary resistance during anti-VEGF therapy (Saharinen, P., et al., Trends Mol Med 17 (2011) 347-362). Both
10 angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) are Tie2-receptor ligands. While Ang1 tends to stabilize and matures blood vessel (Yancopoulos, G. D., et al., Nature 407 (2000) 242-248) Ang2 promotes tumor angiogenesis and growth by destabilizing blood vessels. Ang2 thereby opposes Ang1 in its function (Cascone, T. et al, J Clin Oncol 30 (2012) 441-444). Along this line it has been observed that blocking Ang2, but not Ang1 normalizes tumor blood vessels (Falcon, B. L., H.
15 Hashizume, et al., Am J Pathol 175 (2009) 2159-2170) and helps to overcome acquired resistance towards anti-VEGF therapy (Chae, S. S., W. S. Kamoun, et al., Clin Cancer Res 16 (2010) 3618-3627; Thomas, M., et al. PLoS One 8 (2013) e54923).

20 Members of the ErbB family of receptor tyrosine kinases are important mediators of cell growth, differentiation and survival. The receptor family includes four distinct members, including epidermal growth factor receptor (EGFR or ErbB1), HER2 (ErbB2 or p185^{HER2}), HER3 (ErbB3) and HER4 (ErbB4 or tyro2). HER2 is a transmembrane surface-bound receptor tyrosine kinase and is normally involved in the signal transduction pathways leading to cell growth and differentiation. HER2
25 is a promising target for treatment of breast cancer as it was found to be overexpressed in about one-quarter of breast cancer patients (Bange et al, 2001, Nature Medicine 7:548).

30 The murine monoclonal antibody 4D5 is targeting HER2 specifically in HER2 overexpressing cancer cells, while having no effect on cells expressing physiological levels of HER2. The humanized (4D5) monoclonal antibody (hu4D5)

is commercially known as the drug Herceptin® (trastuzumab, rhuMAb HER2, US Patent No 5,821,337), which gained FDA marketing approval in late 1998.

5 Herceptin was the first monoclonal antibody developed for the treatment of HER2-positive breast cancer and has increased survival times for patients so that they are now the same as for patients with HER2-negative breast cancer. Before Herceptin treatment, shorter survival outcomes were expected for patients diagnosed with HER2-positive breast cancer, compared to patients with HER2-negative disease. In the CLEOPATRA study, PERJETA in combination with Herceptin and chemotherapy has shown the extension of survival times for patients with this
10 aggressive disease even further than Herceptin.

Pertuzumab (PERJETA™, rhuMab 2C4, US Patent No. 7,862,817) is a humanized monoclonal antibody, which is designed specifically to prevent the HER2 receptor from pairing (dimerising) with other HER receptors (EGFR/HER1, HER3 and HER4) on the surface of cells, a process that is believed to play a role in tumor
15 growth and survival. The combination of PERJETA, Herceptin and chemotherapy is thought to provide a more comprehensive blockade of HER signaling pathways. PERJETA is approved in combination with Herceptin (trastuzumab) and docetaxel in adult patients with HER2-positive metastatic or locally recurrent unresectable breast cancer and gained FDA approval for neoadjuvant breast cancer treatment in
20 September 2013. Pertuzumab binds to domain II of HER2, essential for dimerization, while trastuzumab binds to domain IV of HER2.

Li et al (Cancer Research. 2013) describe bispecific, bivalent antibodies to HER2 that overcome trastuzumab resistance. The bispecific, bivalent antibodies described therein are based on the native Trastuzumab and Pertuzumab sequences.

25 WO2010040508, WO2011117329, WO2012131078 relate to bispecific ANG2/VEGF antibodies.

WO2015/091738 relate to improved bispecific antibodies to HER2 that bind to that bind to domain II and domain IV of HER2. The relate to optimizing the native Trastuzumab and Pertuzumab sequences and combining these optimized variants in
30 two different improved bispecific, monovalent antibody formats leading to improved properties as compared to the combination of the monospecific antibodies rhuMab 2C4 and hu 4D5. Further the antibodies are superior to the bivalent antibody formats disclosed in Li et al, as they are monovalent and have the

same molecular weight as the two monospecific antibodies Pertuzumab and Trastuzumab.

Summary of the Invention

5 The present inventors have found that bispecific anti-ANG2/VEGF antibodies enhance the efficacy of bispecific anti-HER2 antibodies to treat cancers or delay progression of a tumor or the survival of a patient afflicted with cancer e.g. with a solid tumor. The delay of progression, the longer survival as well as the potential of reduced doses with lower risk of side effects represent a major benefit for patients.

10 The bispecific anti-HER2 antibodies specifically bind to HER2 comprising a first antigen binding site specific for the domain II of HER2 and a second antigen binding site specific for the domain IV of HER2.

One aspect of the present invention is a bispecific anti-ANG2/VEGF antibody comprising a first antigen binding site specific for ANG2 and a second antigen binding site specific for VEGF; wherein the antibody is administered
15 in combination with a bispecific anti-HER2 antibody comprising a first antigen binding site specific for the domain II of HER2 and a second antigen binding site specific for the domain IV of HER2
a) for use in treating cancer or, b) for use in delaying progression of cancer, or c) for use in prolonging the survival of a patient suffering from cancer.

20 Another aspect of the invention is pharmaceutical kit comprising:

a bispecific anti-ANG2/VEGF antibody comprising a first antigen binding site specific for ANG2 and a second antigen binding site specific for the VEGF;
and a bispecific anti-HER2 antibody comprising a first antigen binding site
25 specific for the domain II of HER2 and a second antigen binding site specific for the domain IV of HER2
wherein the antibodies are to be administered in combination a) for use in treating cancer, or b) for use in delaying progression of cancer, or c) for use in prolonging the survival of a patient suffering from cancer.

30 In one embodiment of the invention the bispecific anti-ANG2/VEGF antibody is monovalent for both ANG2 and VEGF and the bispecific anti-HER2 antibody is monovalent for both the domain II and IV of HER2.

In one embodiment of the invention the bispecific anti-ANG2/VEGF antibody and the bispecific anti-HER2 antibody are both IgG antibodies.

In one embodiment of the invention

i) the bispecific anti-ANG2/VEGF antibody comprises

- 5 a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:1 and a light chain variable comprising domain amino acid sequence of SEQ ID NO:2; and
 a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:5 and a light chain variable domain comprising amino acid sequence of
10 SEQ ID NO:6;

and

ii) the bispecific anti-HER2 antibody comprises

- a heavy chain variable domain comprising amino acid sequence of SEQ ID NO: 11;
15 a heavy chain variable domain comprising amino acid sequence of SEQ ID NO: 12, and
 two light chain variable domains comprising amino acid sequence of SEQ ID NO: 13.

In one embodiment of the invention

20 i) the bispecific anti-ANG2/VEGF antibody comprises

- a heavy chain comprising amino acid sequence SEQ ID NO: 7;
 a heavy chain comprising amino acid sequence SEQ ID NO: 8;
 a light chain comprising amino acid sequence SEQ ID NO: 9; and
 a light chain comprising amino acid sequence SEQ ID NO: 10;

25 and

ii) the bispecific anti-HER2 antibody comprises

- a heavy chain comprising amino acid sequence SEQ ID NO: 14;
 a heavy chain comprising amino acid sequence SEQ ID NO: 15; and
 two light chains comprising amino acid sequence SEQ ID NO: 16.

30 In one embodiment of the invention the cancer is a solid tumor.

In one embodiment of the invention the cancer is further characterized by HER2 expression. In one embodiment of the invention the cancer is further characterized by HER2 overexpression.

5 Surprisingly a treatment of tumors with bispecific anti-ANG2/VEGF antibodies in combination with bispecific anti-HER2 showed strong synergistic effect on the time to tumor progression and the time of survival

Description of the Figures

10 **Figure 1** In vivo anti-tumor efficacy of bispecific anti-ANG2/VEGF antibody in combination with bispecific anti-HER2 domainII/domainIV antibody

Detailed Description of the Invention

Human angiopoietin-2 (ANG-2) (alternatively abbreviated with ANGPT2 or ANG2) (SEQ ID No: 15) is described in Maisonpierre, P.C., et al., Science 277 (1997) 55-60 and Cheung, A.H., et al, Genomics 48 (1998) 389-91. The
15 angiopoietins-1 and -2 (ANG-1(SEQ ID No: 108) and ANG-2 (SEQ ID No: 107) were discovered as ligands for the Ties, a family of tyrosine kinases that is selectively expressed within the vascular endothelium. Yancopoulos, G.D., et al., Nature 407 (2000) 242-48. There are now four definitive members of the angiopoietin family. Angiopoietin-3 and -4 (Ang-3 and Ang-4) may represent
20 widely diverged counterparts of the same gene locus in mouse and man. Kim, I., et al., FEBS Lett, 443 (1999) 353-56; Kim, I., et al., J Biol Chem 274 (1999) 26523-28. ANG-1 and ANG-2 were originally identified in tissue culture experiments as agonist and antagonist, respectively (see for ANG-1: Davies, S., et al., Cell, 87 (1996) 1161-1169; and for ANG-2: Maisonpierre, P.C., et al., Science 277 (1997) 55-60). All of the known angiopoietins bind primarily to Tie2, and both Ang-1 and
25 -2 bind to Tie2 with an affinity of 3 nM (Kd). Maisonpierre, P.C., et al., Science 277 (1997) 55-60. Ang-1 was shown to support EC survival and to promote endothelium integrity, Davis, S., et al., Cell, 87 (1996) 1161-1169; Kwak, H.J., et al., FEBS Lett 448 (1999) 249-53; Suri, C., et al., Science 282 (1998) 468-71; Thurston, G., et al., Science 286 (1999) 2511-14; Thurston, G., et al., Nat. Med. 6 (2000) 460-63, whereas ANG-2 had the opposite effect and promoted blood vessel destabilization and regression in the absence of the survival factors VEGF or basic fibroblast growth factor. Maisonpierre, P.C., et al., Science
30 277 (1997) 55-60. However, many studies of ANG-2 function have suggested a

more complex situation. ANG-2 might be a complex regulator of vascular remodeling that plays a role in both vessel sprouting and vessel regression. Supporting such roles for ANG-2, expression analyses reveal that ANG-2 is rapidly induced, together with VEGF, in adult settings of angiogenic sprouting, whereas
5 ANG-2 is induced in the absence of VEGF in settings of vascular regression. Holash, J., et al., Science 284 (1999) 1994-98; Holash, J., et al., Oncogene 18 (1999) 5356-62. Consistent with a context-dependent role, ANG-2 specifically binds to the same endothelial-specific receptor, Tie-2, which is activated by Ang-1, but has context-dependent effects on its activation. Maisonpierre, P.C., et al.,
10 Science 277 (1997) 55-60.

The term "human ANG2" refers to the human protein angiopoietin 2 (SEQ ID NO: 15). As used herein, "binding to human ANG2" or "specifically binding to human ANG2" or "which binds to human ANG2" or "anti- ANG2 antibody" refers to an antibody specifically binding to the human ANG2 antigen with a binding
15 affinity of KD-value of 1.0×10^{-8} mol/l or lower, in one embodiment of a KD-value of 1.0×10^{-9} mol/l or lower. The binding affinity is determined with a standard binding assay, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden). Thus an "antibody binding to human ANG2" as used herein refers to an antibody specifically binding to the human ANG2 antigen
20 with a binding affinity of KD 1.0×10^{-8} mol/l or lower (in one embodiment 1.0×10^{-8} mol/l - 1.0×10^{-13} mol/l), in one embodiment of a KD 1.0×10^{-9} mol/l or lower (in one embodiment 1.0×10^{-9} mol/l - 1.0×10^{-13} mol/l).

Typically, antibodies bispecific anti-ANG2/VEGF antibody that binds to human
25 ANG-2 and that binds to human VEGF which are useful for the treatment described herein are e.g. disclosed and described in detail in WO2010/040508, WO 2011/117329 or WO2012/131078. Also the VH and VL of the ANG2 antibodies described in WO2010/069532; WO2011/014469; US2011/150895; WO2009/097325; WO2009/105269; WO 2006/068953 or WO 03/030833 can be used within the bispecific
30 antibody using the anti-VEGF binding arms and structures described in WO2010/040508, WO 2011/117329 or WO2012/131078.

In one preferred embodiment, the bispecific antibody that binds to human ANG-2
and that binds to human VEGF comprises
a heavy chain variable domain amino acid sequence of SEQ ID NO:1 and a
35 light chain variable domain amino acid sequence of SEQ ID NO:2; and

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a heavy chain variable domain amino acid sequence of SEQ ID NO:5 and a light chain variable domain amino acid sequence of SEQ ID NO:6.

5 In one preferred embodiment the bispecific antibody that binds to human ANG-2 and that binds to human VEGF comprises the amino acid sequences of SEQ ID NO: 7, of SEQ ID NO: 8, of SEQ ID NO: 9, and of SEQ ID NO: 10.

10 Members of the ErbB family of receptor tyrosine kinases are important mediators of cell growth, differentiation and survival. The receptor family includes four distinct members, including epidermal growth factor receptor (EGFR or ErbB1), HER2 (ErbB2 or p185^{HER2}), HER3 (ErbB3) and HER4 (ErbB4 or tyro2). HER2 is a transmembrane surface-bound receptor tyrosine kinase and is normally involved in the signal transduction pathways leading to cell growth and differentiation. HER2 is a promising target for treatment of breast cancer as it was found to be overexpressed in about one-quarter of breast cancer patients (Bange et al, 2001, 15 Nature Medicine 7:548). Throughout the disclosure, the terms "ErbB2", "ErbB2 receptor", "c-Erb-B2", and "HER2" are used interchangeably, and, unless otherwise indicated, refer to a native sequence ErbB2 human polypeptide, or a functional derivative thereof. "ber2", "erbb2" and "c-erb-B2" refer to the corresponding human gene.

20 The terms "a bispecific anti-HER2 antibody" and "a bispecific antibody that specifically binds to HER2" are used interchangeably and refer to a bispecific antibody that is capable of binding HER2 on both domains II and IV, respectively, with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting cells expressing HER2. In one embodiment, the 25 extent of binding of a bispecific antibody that specifically binds to HER2 on both domains II and IV to an unrelated, non-HER2 protein is less than about 10% of the binding of the antibody to HER2 as measured, e.g., by an Enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR) based assays (e.g. Biacore) or flow cytometry (FACS). In certain embodiments, a bispecific 30 antibody that specifically binds to HER2 has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M).

Typical bispecific anti-HER2 antibody that binds to the domain II of human HER2 and that binds to the domain IV of human HER2 which are useful for the

treatment described herein are e.g. disclosed and described in detail in WO2015/091738.

In one preferred embodiment, the bispecific anti-HER2 antibody that binds to the domain II of human HER2 and that binds to the domain IV of human HER2 comprises
5 a heavy chain variable domain comprising amino acid sequence of SEQ ID NO: 11;
a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:12, and
10 two light chain variable domains comprising amino acid sequence of SEQ ID NO: 13.

In one preferred embodiment the bispecific anti-HER2 antibody that binds to the domain II of human HER2 and that binds to the domain IV of human HER2 comprises
15 a heavy chain comprising amino acid sequence SEQ ID NO: 14;
a heavy chain comprising amino acid sequence SEQ ID NO: 15; and
two light chains comprising amino acid sequence SEQ ID NO: 16.

The term “the bispecific anti-ANG2/VEGF antibody is monovalent for both ANG2 and VEGF” refers to a bispecific antibody with only one antigen binding site for
20 ANG2 and only one antigen binding site for VEGF.

The term “the bispecific anti-HER2 antibody is monovalent for both the domain II and IV of HER2” refers to a bispecific antibody with only one antigen binding site for the domain II of HER2 and only one antigen binding site for the domain IV of HER2.

25 The term “epitope” denotes a protein determinant of the antigen capable of specifically binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually epitopes have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes
30 are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

The “variable domain” (light chain variable domain VL, heavy chain variable domain VH) as used herein denotes each of the pair of light and heavy chain

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

The term “antigen-binding site of an antibody” when used herein refer to the amino acid residues of an antibody which are responsible for antigen-binding. The antigen-binding site of an antibody comprises amino acid residues from the “complementary determining regions” or “CDRs”. In one embodiment of the invention the antigen binding site comprises a heavy chain variable domain, in one preferred embodiment of the invention the antigen binding site comprise a heavy chain variable domain and a light chain variable domain (both forming together the antigen binding site).

“Framework” or “FR” regions are those variable domain regions other than the hypervariable region residues as herein defined. Therefore, the light and heavy chain variable domains of an antibody comprise from N- to C-terminus the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. Especially, CDR3 of the heavy chain is the region which contributes most to antigen binding and defines the antibody’s properties. CDR and FR regions are determined according to the standard definition of Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and/or those residues from a “hypervariable loop”.

The terms “nucleic acid” or “nucleic acid molecule”, as used herein, are intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term “amino acid” as used within this application denotes the group of naturally occurring carboxy alpha-amino acids comprising alanine (three letter code: ala, one letter code: A), arginine (arg, R), asparagine (asn, N), aspartic acid

(asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V).

5 The “Fc part” of an antibody is not involved directly in binding of an antibody to an antigen, but exhibit various effector functions. A “Fc part of an antibody” is a term well known to the skilled artisan and defined on the basis of papain cleavage of antibodies. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies or immunoglobulins are divided in the classes: IgA, IgD,
10 IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG1, IgG2, IgG3, and IgG4, IgA1, and IgA2. According to the heavy chain constant regions the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The Fc part of an antibody is directly involved in ADCC (antibody-dependent cell-mediated cytotoxicity) and CDC (complement-
15 dependent cytotoxicity) based on complement activation, C1q binding and Fc receptor binding. Complement activation (CDC) is initiated by binding of complement factor C1q to the Fc part of most IgG antibody subclasses. While the influence of an antibody on the complement system is dependent on certain conditions, binding to C1q is caused by defined binding sites in the Fc part. Such
20 binding sites are known in the state of the art and described e.g. by Boackle, R.J., et al., Nature 282 (1979) 742-743; Lukas, T.J., et al., J. Immunol. 127 (1981) 2555-2560; Brunhouse, R., and Cebra, J.J., Mol. Immunol. 16 (1979) 907-917; Burton, D.R., et al., Nature 288 (1980) 338-344; Thommesen, J.E., et al., Mol. Immunol. 37 (2000) 995-1004; Idusogie, E.E., et al., J. Immunol. 164 (2000) 4178-4184;
25 Hezareh, M., et al., J. Virology 75 (2001) 12161-12168; Morgan, A., et al., Immunology 86 (1995) 319-324; EP 0 307 434. Such binding sites are e.g. L234, L235, D270, N297, E318, K320, K322, P331 and P329 (numbering according to EU index of Kabat, E.A., see below). Antibodies of subclass IgG1, IgG2 and IgG3 usually show complement activation and C1q and C3 binding, whereas IgG4 do not
30 activate the complement system and do not bind C1q and C3.

In one embodiment the antibodies described herein are of human IgG class (i.e. of IgG1, IgG2, IgG3 or IgG4 subclass).

In a preferred embodiment the antibodies described herein are of human IgG1 subclass or of human IgG4 subclass. In one embodiment the described herein are of

human IgG1 subclass. In one embodiment the antibodies described herein are of human IgG4 subclass.

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

In one embodiment the antibody described herein is characterized in that the constant chains are of human origin. Such constant chains are well known in the state of the art and e.g. described by Kabat, E.A., (see e.g. Johnson, G. and Wu, T.T., Nucleic Acids Res. 28 (2000) 214-218).

15 The invention comprises a method for the treatment of a patient in need of therapy, characterized by administering to the patient a therapeutically effective amount of the bispecific anti-ANG2/VEGF antibody described herein in combination with administering a therapeutically effective amount of a bispecific anti-HER2 antibody described herein.

20 The invention comprises the use of the bispecific anti-ANG2/VEGF antibody described herein in combination with a bispecific anti-HER2 antibody as described herein for the described therapy.

The antibodies described herein are preferably produced by recombinant means. Such methods are widely known in the state of the art and comprise protein
25 expression in prokaryotic and eukaryotic cells with subsequent isolation of the antibody polypeptide and usually purification to a pharmaceutically acceptable purity. For the protein expression nucleic acids encoding light and heavy chains or fragments thereof are inserted into expression vectors by standard methods. Expression is performed in appropriate prokaryotic or eukaryotic host cells, such as
30 CHO cells, NS0 cells, SP2/0 cells, HEK293 cells, COS cells, yeast, or E. coli cells, and the antibody is recovered from the cells (from the supernatant or after cells lysis).

Recombinant production of antibodies is well-known in the state of the art and described, for example, in the review articles of Makrides, S.C., *Protein Expr. Purif.* 17 (1999) 183-202; Geisse, S., et al., *Protein Expr. Purif.* 8 (1996) 271-282; Kaufman, R.J., *Mol. Biotechnol.* 16 (2000) 151-161; Werner, R.G., *Drug Res.* 48 (1998) 870-880.

The antibodies may be present in whole cells, in a cell lysate, or in a partially purified, or substantially pure form. Purification is performed in order to eliminate other cellular components or other contaminants, e.g. other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis, and others well known in the art. See Ausubel, F., et al., ed. *Current Protocols in Molecular Biology*, Greene Publishing and Wiley Interscience, New York (1987).

Expression in NS0 cells is described by, e.g., Barnes, L.M., et al., *Cytotechnology* 32 (2000) 109-123; Barnes, L.M., et al., *Biotech. Bioeng.* 73 (2001) 261-270. Transient expression is described by, e.g., Durocher, Y., et al., *Nucl. Acids. Res.* 30 (2002) E9. Cloning of variable domains is described by Orlandi, R., et al., *Proc. Natl. Acad. Sci. USA* 86 (1989) 3833-3837; Carter, P., et al., *Proc. Natl. Acad. Sci. USA* 89 (1992) 4285-4289; Norderhaug, L., et al., *J. Immunol. Methods* 204 (1997) 77-87. A preferred transient expression system (HEK 293) is described by Schlaeger, E.-J. and Christensen, K., in *Cytotechnology* 30 (1999) 71-83, and by Schlaeger, E.-J., in *J. Immunol. Methods* 194 (1996) 191-199.

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

The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, enhancers and polyadenylation signals.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a

preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The monoclonal antibodies are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. DNA and RNA encoding the monoclonal antibodies are readily isolated and sequenced using conventional procedures. The hybridoma cells can serve as a source of such DNA and RNA. Once isolated, the DNA may be inserted into expression vectors, which are then transfected into host cells such as HEK 293 cells, CHO cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of recombinant monoclonal antibodies in the host cells.

As used herein, the expressions "cell", "cell line", and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

In another aspect, the present invention provides a composition, e.g. a pharmaceutical composition, containing one or a combination of monoclonal antibodies, or the antigen-binding portion thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption/resorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for injection or infusion.

A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

5 Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. In addition to water, the carrier can be, for example, an isotonic buffered saline solution.

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

15 Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient (effective amount). The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of
20 the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and
25 like factors well known in the medical arts.

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

the medical history and estimated survival expectancy of a patient, is nevertheless deemed to induce an overall beneficial course of action.

5 The terms “administered in combination with” or “co-administration”, “co-administering”, “combination therapy”, “administered with” or “combination treatment” refer to the administration of the bispecific anti-ANG2/VEGF antibody as described herein, and the bispecific anti-HER2 antibody as described herein e.g. as separate formulations/applications (or as one single formulation/application). The co-administration can be simultaneous or sequential in either order, wherein preferably there is a time period while both (or all) active agents simultaneously
10 exert their biological activities. Said antibody and said further agent are co-administered either simultaneously or sequentially (e.g. intravenous (i.v.) through a continuous infusion. When both therapeutic agents are co-administered sequentially the dose is administered either on the same day in two separate administrations, or one of the agents is administered on day 1 and the second is co-administered on day 2 to day 7, preferably on day 2 to 4. Thus in one embodiment the term “sequentially” means within 7 days after the dose of the first component, preferably within 4 days after the dose of the first component; and the term “simultaneously” means at the same time. The terms “co-administration” with respect to the maintenance doses of bispecific anti-ANG2/VEGF antibody and/or
20 bispecific anti-HER2 antibody mean that the maintenance doses can be either co-administered simultaneously, if the treatment cycle is appropriate for both drugs, e.g. every week. Or the further agent is e.g. administered e.g. every first to third day and said antibody is administered every week. Or the maintenance doses are co-administered sequentially, either within one or within several days.

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

30 As used herein, the term “patient” or “subject” preferably refers to a human in need of treatment of cancer, or a precancerous condition or lesion. However, the term “patient” can also refer to non-human animals, e.g. mammals such as mice, dogs, cats, horses, cows, pigs, sheep and non-human primates, among others, that are in need of treatment.

The amount of co-administration and the timing of co-administration will depend on the type (species, gender, age, weight, etc.) and condition of the patient being treated and the severity of the disease or condition being treated. Said bispecific anti-ANG2/VEGF antibody and further agent are suitably co-administered to the patient at one time or over a series of treatments e.g. on the same day or on the day after.

Depending on the type and severity of the disease, about 0.1 mg /kg to 50 mg/kg (e.g. 0.1-20 mg/kg) of said bispecific anti-ANG2/VEGF antibody and/or bispecific anti-HER2 antibody; is an initial candidate dosage for co-administration of both drugs to the patient The invention comprises the use of the antibodies according to the invention for the treatment of a patient suffering from cancer, especially from colon cancer, ovarian cancer, glioblastoma, gastric cancer, pancreatic cancer, breast cancer, lung cancer, hepatocellular cancer.

In addition to the bispecific anti-ANG2/VEGF antibody in combination with the bispecific anti-HER2 antibody also a chemotherapeutic agent can be administered.

In one embodiment such additional chemotherapeutic agents, which may be administered with bispecific anti-ANG2/VEGF antibody as described herein and the bispecific anti-HER2 antibody as described herein , include, but are not limited to, anti-neoplastic agents including alkylating agents including: nitrogen mustards, such as mechlorethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU); Temodal(TM) (temozolamide), ethylenimines/methylmelamine such as triethylenemelamine (TEM), triethylene, thiophosphoramidate (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil (5FU), fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2'-difluorodeoxycytidine, purine analogs such as 6-mercaptopurine, 6-thioguanine, azathioprine, T-deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2- chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and vinorelbine, taxotere, estramustine, and estramustine phosphate; pipodophylotoxins such as etoposide and teniposide; antibiotics such as actinomycin D, daunomycin (rubidomycin), doxorubicin,

mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycin C, and actinomycin; enzymes such as L-asparaginase; biological response modifiers such as interferon-alpha, IL-2, G-CSF and GM-CSF; miscellaneous agents including platinum coordination complexes such as oxaliplatin, cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N- methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o, p-DDD) and aminogluthethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminogluthethimide; Gemzar(TM) (gemcitabine), progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; and non-steroidal antiandrogens such as flutamide. Therapies targeting epigenetic mechanism including, but not limited to, histone deacetylase inhibitors, demethylating agents (e.g., Vidaza) and release of transcriptional repression (ATRA) therapies can also be combined with the antigen binding proteins. In one embodiment the chemotherapeutic agent is selected from the group consisting of taxanes (like e.g. paclitaxel (Taxol), docetaxel (Taxotere), modified paclitaxel (e.g., Abraxane and Opaxio), doxorubicin, sunitinib (Sutent), sorafenib (Nexavar), and other multikinase inhibitors, oxaliplatin, cisplatin and carboplatin, etoposide, gemcitabine, and vinblastine. In one embodiment the chemotherapeutic agent is selected from the group consisting of taxanes (like e.g. taxol (paclitaxel), docetaxel (Taxotere), modified paclitaxel (e.g. Abraxane and Opaxio). In one embodiment, the additional chemotherapeutic agent is selected from 5-fluorouracil (5-FU), leucovorin, irinotecan, or oxaliplatin. In one embodiment the chemotherapeutic agent is 5-fluorouracil, leucovorin and irinotecan (FOLFIRI). In one embodiment the chemotherapeutic agent is 5-fluorouracil, and oxaliplatin (FOLFOX).

Specific examples of combination therapies with additional chemotherapeutic agents include, for instance, therapies taxanes (e.g., docetaxel or paclitaxel) or a modified paclitaxel (e.g., Abraxane or Opaxio), doxorubicin, capecitabine and/or bevacizumab (Avastin) for the treatment of breast cancer; therapies with carboplatin, oxaliplatin, cisplatin, paclitaxel, doxorubicin (or modified doxorubicin (Caelyx or Doxil)), or topotecan (Hycamtin) for ovarian cancer, the therapies with

a multi-kinase inhibitor, MKI, (Sutent, Nexavar, or 706) and/or doxorubicin for treatment of kidney cancer; therapies with oxaliplatin, cisplatin and/or radiation for the treatment of squamous cell carcinoma; therapies with taxol and/or carboplatin for the treatment of lung cancer.

5 Therefore, in one embodiment the additional chemotherapeutic agent is selected from the group of taxanes (docetaxel or paclitaxel or a modified paclitaxel (Abraxane or Opaxio), doxorubicin, capecitabine and/or bevacizumab for the treatment of breast cancer.

10 In one embodiment of the bispecific anti-ANG2/VEGF antibody/ bispecific anti-HER2 antibody combination therapy, no additional chemotherapeutic agents are administered.

The invention comprises also a method for the treatment of a patient suffering from such disease.

15 The invention further provides a method for the manufacture of a pharmaceutical composition comprising an effective amount of an antibody according to the invention together with a pharmaceutically acceptable carrier and the use of the antibody according to the invention for such a method.

20 The invention further provides the use of an antibody according to the invention in an effective amount for the manufacture of a pharmaceutical agent, preferably together with a pharmaceutically acceptable carrier, for the treatment of a patient suffering from cancer.

25 The invention also provides the use of an antibody according to the invention in an effective amount for the manufacture of a pharmaceutical agent, preferably together with a pharmaceutically acceptable carrier, for the treatment of a patient suffering from cancer.

Description of the amino acid sequences

SEQ ID NO: 1	variable heavy chain domain VH of <ANG-2> E6Q
SEQ ID NO: 2	variable light chain domain VL of <ANG-2> E6Q
SEQ ID NO: 3	variable heavy chain domain VH of <ANG-2> Ang2i_LC06
30 SEQ ID NO: 4	variable light chain domain VL of <ANG-2> Ang2i_LC06
SEQ ID NO: 5	variable heavy chain domain VH of <VEGF> bevacizumab
SEQ ID NO: 6	variable light chain domain VL of <VEGF> bevacizumab

	SEQ ID NO: 7	bispecific anti-ANG2/VEGF antibody heavy chain 1
	SEQ ID NO: 8	bispecific anti-ANG2/VEGF antibody heavy chain 2
	SEQ ID NO: 9	bispecific anti-ANG2/VEGF antibody light chain 1
	SEQ ID NO: 10	bispecific anti-ANG2/VEGF antibody light chain 2
5	SEQ ID NO: 11	variable heavy chain domain VH1 of <HER2>
	SEQ ID NO: 12	variable heavy chain domain VH2 of <HER2>
	SEQ ID NO: 13	variable light chain domain VL of <HER2>
	SEQ ID NO: 14	bispecific anti-HER2 antibody heavy chain 1
	SEQ ID NO: 15	bispecific anti-HER2 antibody heavy chain 2
10	SEQ ID NO: 16	bispecific anti-HER2 antibody light chain
	SEQ ID NO: 17	exemplary human angiopoietin-2 (ANG-2)
	SEQ ID NO: 18	exemplary human vascular endothelial growth factor (VEGF)
	SEQ ID NO: 19	exemplary human HER2
	SEQ ID NO: 20	exemplary human HER2 domain II
15	SEQ ID NO: 21	exemplary human HER2 domain IV
	SEQ ID NO: 22	variable heavy chain domain VH of <VEGF> B20-4.1
	SEQ ID NO: 23	variable light chain domain VL of <VEGF> B20-4.1

In the following specific embodiments of the invention are described:

1. A bispecific anti-ANG2/VEGF antibody comprising a first antigen binding site specific for ANG2 and a second antigen binding site specific for the VEGF; wherein the antibody is administered in combination with a bispecific anti-HER2 antibody comprising a first antigen binding site specific for the domain II of HER2 and a second antigen binding site specific for the domain IV of HER2
 - a) for use in treating cancer, or b) for use in delaying progression of cancer, or c) for use in prolonging the survival of a patient suffering from cancer.
2. The antibody for use according to embodiment 1, wherein the bispecific anti-ANG2/VEGF antibody is monovalent for both ANG2 and VEGF, wherein the bispecific anti-HER2 antibody is monovalent for both the domain II and IV of HER2.
3. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF antibody and the bispecific anti-HER2 antibody are both IgG antibodies.

4. The antibody for use according to any one of the preceding embodiments
- i) wherein the bispecific anti-ANG2/VEGF antibody comprises
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO:1 and a light chain variable comprising domain amino acid sequence of
5 SEQ ID NO:2; and
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO:5 and a light chain variable domain comprising amino acid sequence of
SEQ ID NO:6;
and
- 10 ii) wherein the bispecific anti-HER2 antibody comprises
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO: 11;
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO:12, and
15 two light chain variable domains comprising amino acid sequence of SEQ ID
NO: 13.
5. The antibody for use according to any one of the preceding embodiments
- i) wherein the bispecific anti-ANG2/VEGF antibody comprises
a heavy chain comprising amino acid sequence SEQ ID NO: 7;
20 a heavy chain comprising amino acid sequence SEQ ID NO: 8;
a light chain comprising amino acid sequence SEQ ID NO: 9; and
a light chain comprising amino acid sequence SEQ ID NO: 10;
and
- 25 ii) wherein the bispecific anti-HER2 antibody comprises
a heavy chain comprising amino acid sequence SEQ ID NO: 14;
a heavy chain comprising amino acid sequence SEQ ID NO: 15; and
two light chains comprising amino acid sequence SEQ ID NO: 16.
6. The antibody for use according to any one of the preceding embodiments,
wherein the cancer is lung cancer, non-small cell lung (NSCL) cancer,
30 bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin
cancer, cancer of the head or neck, cutaneous or intraocular melanoma,
uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region,

- 5 stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma, lymphoma or lymphocytic leukemia.
- 10
7. The antibody for use according to embodiment 6, wherein the cancer is a solid tumor.
- 15
8. The antibody for use according to embodiments 6 or 7, wherein the cancer is further characterized by HER2 expression (in embodiment by HER2 overexpression).
9. The antibody for use according to any one of embodiments 6 to 8, wherein the cancer is colon cancer, ovarian cancer, glioblastoma, gastric cancer, pancreatic cancer, breast cancer, lung cancer, hepatocellular cancer.
- 20
10. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered simultaneously.
- 25
11. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered sequentially.
12. A pharmaceutical kit comprising:
- 30 a bispecific anti-ANG2/VEGF antibody comprising a first antigen binding site specific for ANG2 and a second antigen binding site specific for the VEGF;
and a bispecific anti-HER2 antibody comprising a first antigen binding site

specific for the domain II of HER2 and a second antigen binding site specific for the domain IV of HER2

wherein the antibodies are to be administered in combination a) for use in treating cancer, or b) for use in delaying progression of cancer, or c) for use in prolonging the survival of a patient suffering from cancer.

5

13. The kit according to embodiment 12, wherein the bispecific anti-ANG2/VEGF antibody is monovalent for both ANG2 and VEGF, wherein the bispecific anti-HER2 antibody is monovalent for both the domain II and IV of HER2.

10

14. The kit according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF antibody and the bispecific anti-HER2 antibody are both IgG antibodies.

15. The kit according to any one of the preceding embodiments

15

i) wherein the bispecific anti-ANG2/VEGF antibody comprises a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:1 and a light chain variable comprising domain amino acid sequence of SEQ ID NO:2; and

a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:5 and a light chain variable domain comprising amino acid sequence of SEQ ID NO:6;

20

and

ii) wherein the bispecific anti-HER2 antibody comprises a heavy chain variable domain comprising amino acid sequence of SEQ ID NO: 11;

25

a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:12, and two light chain variable domains comprising amino acid sequence of SEQ ID NO: 13.

16. The kit according to any one of the preceding embodiments

30

i) wherein the bispecific anti-ANG2/VEGF antibody comprises a heavy chain comprising amino acid sequence SEQ ID NO: 7; a heavy chain comprising amino acid sequence SEQ ID NO: 8;

a light chain comprising amino acid sequence SEQ ID NO: 9; and
a light chain comprising amino acid sequence SEQ ID NO: 10;

and

ii) wherein the bispecific anti-HER2 antibody comprises

- 5 a heavy chain comprising amino acid sequence SEQ ID NO: 14;
a heavy chain comprising amino acid sequence SEQ ID NO: 15; and
two light chains comprising amino acid sequence SEQ ID NO: 16.

- 10 17. The kit according to any one of the preceding embodiments, wherein the
bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are to be
administered simultaneously.
18. The kit according to any one of the preceding embodiments, wherein the
bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are to be
administered sequentially.

In the following specific embodiments of the invention are described:

- 15 1. A bispecific anti-HER2 antibody comprising a first antigen binding site
specific for the domain II of HER2 and a second antigen binding site specific
for the domain IV of HER2; wherein the antibody is administered in
combination with a bispecific anti-ANG2/VEGF comprising a first antigen
binding site specific for ANG2 and a second antigen binding site specific for
20 the VEGF
a) for use in treating cancer, or b) for use in delaying progression of cancer,
or c) for use in prolonging the survival of a patient suffering from cancer.
2. The antibody for use according to embodiment 1, wherein the bispecific anti-
ANG2/VEGF antibody is monovalent for both ANG2 and VEGF, wherein
25 the bispecific anti-HER2 antibody is monovalent for both the domain II and
IV of HER2.
3. The antibody for use according to any one of the preceding embodiments,
wherein the bispecific anti-ANG2/VEGF antibody and the bispecific anti-
HER2 antibody are both IgG antibodies.

4. The antibody for use according to any one of the preceding embodiments
- 5 i) wherein the bispecific anti-ANG2/VEGF antibody comprises
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO:1 and a light chain variable domain comprising amino acid sequence of
SEQ ID NO:2; and
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO:5 and a light chain variable domain comprising amino acid sequence of
SEQ ID NO:6;
and
- 10 ii) wherein the bispecific anti-HER2 antibody comprises
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO: 11;
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO:12, and
15 two light chain variable domains comprising amino acid sequence of SEQ ID
NO: 13.
5. The antibody for use according to any one of the preceding embodiments
- 20 i) wherein the bispecific anti-ANG2/VEGF antibody comprises
a heavy chain comprising amino acid sequence SEQ ID NO: 7;
a heavy chain comprising amino acid sequence SEQ ID NO: 8;
a light chain comprising amino acid sequence SEQ ID NO: 9; and
a light chain comprising amino acid sequence SEQ ID NO: 10;
and
- 25 ii) wherein the bispecific anti-HER2 antibody comprises
a heavy chain comprising amino acid sequence SEQ ID NO: 14;
a heavy chain comprising amino acid sequence SEQ ID NO: 15; and
two light chains comprising amino acid sequence SEQ ID NO: 16.
6. The antibody for use according to any one of the preceding embodiments,
wherein the cancer is lung cancer, non small cell lung (NSCL) cancer,
30 bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin
cancer, cancer of the head or neck, cutaneous or intraocular melanoma,
uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region,

- 5 stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma, lymphoma or lymphocytic leukemia.
- 10
7. The antibody for use according to embodiment 6, wherein the cancer is a solid tumor.
- 15
8. The antibody for use according to embodiments 6 or 7, wherein the cancer is further characterized by HER2 expression (in embodiment by HER2 overexpression).
9. The antibody for use according to any one of embodiments 6 to 8, wherein the cancer is colon cancer, ovarian cancer, glioblastoma, gastric cancer, pancreatic cancer, breast cancer, lung cancer, hepatocellular cancer.
- 20
10. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered simultaneously.
- 25
11. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered sequentially.

In the following specific embodiments of the invention are described:

1. A bispecific anti-ANG2/VEGF antibody comprising a first antigen binding site specific for ANG2 and a second antigen binding site specific for the VEGF and a bispecific anti-HER2 antibody comprising a first antigen binding site specific for the domain II of HER2 and a second antigen binding
- 30

site specific for the domain IV of HER2; wherein the antibodies are administered in combination

a) for use in treating cancer , or b) for use in delaying progression of cancer, or c) for use in prolonging the survival of a patient suffering from cancer.

- 5 2. The antibody for use according to embodiment 1, wherein the bispecific anti-ANG2/VEGF antibody is monovalent for both ANG2 and VEGF, wherein the bispecific anti-HER2 antibody is monovalent for both the domain II and IV of HER2.
- 10 3. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF antibody and the bispecific anti-HER2 antibody are both IgG antibodies.
- 15 4. The antibody for use according to any one of the preceding embodiments
i) wherein the bispecific anti-ANG2/VEGF antibody comprises
a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:1 and a light chain variable comprising domain amino acid sequence of SEQ ID NO:2; and
a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:5 and a light chain variable domain comprising amino acid sequence of SEQ ID NO:6;
20 and
ii) wherein the bispecific anti-HER2 antibody comprises
a heavy chain variable domain comprising amino acid sequence of SEQ ID NO: 11;
a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:12, and
25 two light chain variable domains comprising amino acid sequence of SEQ ID NO: 13.
- 30 5. The antibody for use according to any one of the preceding embodiments
i) wherein the bispecific anti-ANG2/VEGF antibody comprises
a heavy chain comprising amino acid sequence SEQ ID NO: 7;
a heavy chain comprising amino acid sequence SEQ ID NO: 8;
a light chain comprising amino acid sequence SEQ ID NO: 9; and

a light chain comprising amino acid sequence SEQ ID NO: 10;

and

ii) wherein the bispecific anti-HER2 antibody comprises

a heavy chain comprising amino acid sequence SEQ ID NO: 14;

5 a heavy chain comprising amino acid sequence SEQ ID NO: 15; and

two light chains comprising amino acid sequence SEQ ID NO: 16.

6. The antibody for use according to any one of the preceding embodiments,
wherein the cancer is lung cancer, non small cell lung (NSCL) cancer,
bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin
10 cancer, cancer of the head or neck, cutaneous or intraocular melanoma,
uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region,
stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer,
carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma
of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's
15 Disease, cancer of the esophagus, cancer of the small intestine, cancer of the
endocrine system, cancer of the thyroid gland, cancer of the parathyroid
gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the
urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of
the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis,
20 mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central
nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma
multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas,
meningiomas, squamous cell carcinomas, pituitary adenoma, lymphoma or
lymphocytic leukemia.

25 7. The antibody for use according to embodiment 6, wherein the cancer is a
solid tumor.

8. The antibody for use according to embodiments 6 or 7, wherein the cancer is
further characterized by HER2 expression (in embodiment by HER2
overexpression).

30 9. The antibody for use according to any one of embodiments 6 to 8, wherein
the cancer is colon cancer, ovarian cancer, glioblastoma, gastric cancer,
pancreatic cancer, breast cancer, lung cancer, hepatocellular cancer.

10. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered simultaneously.

5 11. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered sequentially.

In the following specific embodiments of the invention are described:

1. Use of a bispecific anti-ANG2/VEGF antibody comprising a first antigen binding site specific for ANG2 and a second antigen binding site specific for the VEGF; for the manufacture of a medicament a) for use in treating cancer, or b) for use in delaying progression of cancer, or b) for use in prolonging the survival of a patient suffering from cancer; wherein the antibody is administered in combination with a bispecific anti-HER2 antibody comprising a first antigen binding site specific for the domain II of HER2 and a second antigen binding site specific for the domain IV of HER2.

2. The use according to embodiment 1, wherein the bispecific anti-ANG2/VEGF antibody is monovalent for both ANG2 and VEGF, wherein the bispecific anti-HER2 antibody is monovalent for both the domain II and IV of HER2.

3. The use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF antibody and the bispecific anti-HER2 antibody are both IgG antibodies.

4. The use according to any one of the preceding embodiments

25 i) wherein the bispecific anti-ANG2/VEGF antibody comprises a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:1 and a light chain variable comprising domain amino acid sequence of SEQ ID NO:2; and a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:5 and a light chain variable domain comprising amino acid sequence of SEQ ID NO:6;

30 and

- ii) wherein the bispecific anti-HER2 antibody comprises
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO: 11;
a heavy chain variable domain comprising amino acid sequence of SEQ ID
NO:12, and
two light chain variable domains comprising amino acid sequence of SEQ ID
NO: 13.
- 5
5. The use according to any one of the preceding embodiments
- i) wherein the bispecific anti-ANG2/VEGF antibody comprises
a heavy chain comprising amino acid sequence SEQ ID NO: 7;
a heavy chain comprising amino acid sequence SEQ ID NO: 8;
a light chain comprising amino acid sequence SEQ ID NO: 9; and
a light chain comprising amino acid sequence SEQ ID NO: 10;
- 10
- and
- ii) wherein the bispecific anti-HER2 antibody comprises
a heavy chain comprising amino acid sequence SEQ ID NO: 14;
a heavy chain comprising amino acid sequence SEQ ID NO: 15; and
two light chains comprising amino acid sequence SEQ ID NO: 16.
- 15
6. The use according to any one of the preceding embodiments, wherein the
cancer is lung cancer, non small cell lung (NSCL) cancer, bronchioloalviolar
cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the
head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian
cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric
cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian
tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of
the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the
esophagus, cancer of the small intestine, cancer of the endocrine system,
cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the
adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the
penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter,
renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma,
hepatocellular cancer, biliary cancer, neoplasms of the central nervous system
(CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme,
astrocytomas, schwannomas, ependymomas, medulloblastomas, meningiomas,
- 20
- 25
- 30

squamous cell carcinomas, pituitary adenoma, lymphoma or lymphocytic leukemia.

7. The bispecific anti-ANG2/VEGF for use according to embodiment 6, wherein the cancer is a solid tumor.
- 5 8. The use according to embodiments 6 or 7, wherein the cancer is further characterized by HER2 expression (in embodiment by HER2 overexpression).
9. The use according to any one of embodiments 6 to 8, wherein the cancer is colon cancer, ovarian cancer, glioblastoma, gastric cancer, pancreatic cancer,
10 breast cancer, lung cancer, hepatocellular cancer.
10. The use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered simultaneously.
11. The use according to any one of the preceding embodiments, wherein the
15 bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered sequentially.

In the following embodiments of the invention are described:

1. A method of treatment wherein a bispecific anti-ANG2/VEGF antibody comprising a first antigen binding site specific for ANG2 and a second
20 antigen binding site specific for the VEGF is administered in combination with a bispecific anti-HER2 antibody comprising a first antigen binding site specific for the domain II of HER2 and a second antigen binding site specific for the domain IV of HER2, wherein the treatment is a) for use in treating cancer , or b) for use in delaying progression of cancer, or b) for use in
25 prolonging the survival of a patient suffering from cancer.
2. The method according to embodiment 1, wherein the bispecific anti-ANG2/VEGF antibody is monovalent for both ANG2 and VEGF, wherein the bispecific anti-HER2 antibody is monovalent for both the domain II and IV of HER2.

3. The method according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF antibody and the bispecific anti-HER2 antibody are both IgG antibodies.
4. The method according to any one of the preceding embodiments
 - 5 i) wherein the bispecific anti-ANG2/VEGF antibody comprises a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:1 and a light chain variable comprising domain amino acid sequence of SEQ ID NO:2; and a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:5 and a light chain variable domain comprising amino acid sequence of SEQ ID NO:6;
10 and
ii) wherein the bispecific anti-HER2 antibody comprises a heavy chain variable domain comprising amino acid sequence of SEQ ID NO: 11;
15 a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:12, and two light chain variable domains comprising amino acid sequence of SEQ ID NO: 13.
- 20 5. The method according to any one of the preceding embodiments
 - i) wherein the bispecific anti-ANG2/VEGF antibody comprises a heavy chain comprising amino acid sequence SEQ ID NO: 7;
a heavy chain comprising amino acid sequence SEQ ID NO: 8;
a light chain comprising amino acid sequence SEQ ID NO: 9; and
25 a light chain comprising amino acid sequence SEQ ID NO: 10;
and
ii) wherein the bispecific anti-HER2 antibody comprises a heavy chain comprising amino acid sequence SEQ ID NO: 14;
a heavy chain comprising amino acid sequence SEQ ID NO: 15; and
30 two light chains comprising amino acid sequence SEQ ID NO: 16.

6. The method according to any one of the preceding embodiments, wherein the cancer is lung cancer, non small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma, lymphoma or lymphocytic leukemia.
7. The method according to embodiment 6, wherein the cancer is a solid tumor.
8. The method according to embodiments 6 or 7, wherein the cancer is further characterized by HER2 expression (in embodiment by HER2 overexpression).
9. The method according to any one of embodiments 6 to 8, wherein the cancer is colon cancer, ovarian cancer, glioblastoma, gastric cancer, pancreatic cancer, breast cancer, lung cancer, hepatocellular cancer.
10. The method according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered simultaneously.
11. The method according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered sequentially.

Example 1**Inhibition of the interaction of human ANG-2 with TIE2 receptor (experiment A)**

Blocking of human ANG-2/human Tie2 interaction was shown by receptor interaction ELISA. 384-well Maxisorp plates (Nunc) were coated with 0.5 µg/ml human Tie2 (R&D Systems, UK, Cat.No.313-TI or in house produced material) for 2 h at room temperature and blocked with PBS supplemented with 0.2% Tween-20 and 2% BSA (Roche Diagnostics GmbH, DE) for 1 h at room temperature under shaking. In the meantime, Dilutions of purified antibodies in PBS were incubated together with 0.2 µg/ml huAngiopoietin-1/2 (R&D Systems #923-AN/CF, R&D Systems, UK, Cat.No. 623-AN or in house produced material) for 1 hour at RT. After washing a mixture of 0.5 µg/ml biotinylated anti-Angiopoietin-1/2 clone (R&D Systems #BAF923, BAM0981 R&D Systems, UK) and 1:3000 diluted streptavidin HRP (Roche Diagnostics GmbH, DE, Cat.No.11089153001) was added for 1 h. Thereafter the plates were washed 6 times with PBST. Plates were developed with freshly prepared ABTS reagent (Roche Diagnostics GmbH, DE, buffer #204 530 001, tablets #11 112 422 001) for 30 minutes at RT. Absorbance was measured at 405 nm.

The obtained inhibitory concentrations are summarized in the following table.

Antibody	ANG2/ Tie2 interaction ELISA
Ang2i-LC06	0.1 nM

Inhibition of the interaction of human ANG-2 with TIE2 receptor (experiment B)

The interaction ELISA was performed on 384 well microtiter plates (MicroCoat, DE, Cat.No. 464718) at RT. After each incubation step plates were washed 3 times with PBST. ELISA plates were coated with 5 µg/ml Tie-2 protein for 1 hour (h). Thereafter the wells were blocked with PBS supplemented with 0.2% Tween-20 and 2% BSA (Roche Diagnostics GmbH, DE) for 1 h. Dilutions of purified bispecific X Mab antibodies in PBS were incubated together with 0.2 µg/ml huAngiopoietin-2 (R&D Systems, UK, Cat.No. 623-AN) for 1 h at RT. After washing a mixture of 0.5 µg/ml biotinylated anti-Angiopoietin-2 clone BAM0981 (R&D Systems, UK) and 1:3000 diluted streptavidin HRP (Roche Diagnostics

GmbH, DE, Cat.No.11089153001) was added for 1 h. Thereafter the plates were washed 3 times with PBST. Plates are developed with freshly prepared ABTS reagent (Roche Diagnostics GmbH, DE, buffer #204 530 001, tablets #11 112 422 001) for 30 minutes at RT. Absorbance was measured at 405 nm and the IC50 was determined.

XMab1, a bispecific antibody that binds to human ANG2 and to human VEGF (see WO2011/117329 and sequences SEQ ID NOs:11- 14, showed an inhibition of ANG-2 binding to Tie-2 (ANG2/Tie2 receptor interaction inhibition) with an IC50 of 12 nM.

Example 2

In vivo anti-tumor efficacy of bispecific anti-ANG2/VEGF antibody in combination with bispecific anti-HER2 domainII/domainIV antibody

Methods

Test agents

The bispecific ANG2/VEGF antibody based on the VH and VL of <ANG-2> E6Q (SEQ ID NOs:1-2) for the ANG2 binding arm. As mouse-cross reactive surrogate VEGF binding arm the VH and VL of <VEGF> B20-4.1 (SEQ ID NOs:22-23) for the bispecific antibody were used instead of the VH and VL of <VEGF> bevacizumab (SEQ ID NOs:5-6). The structure, however was the same as that for the bispecific ANG2/VEGF antibody based on the 2 heavy chains and the two light chains of SEQ ID NOs:7-10 (only VH/VLs <VEGF> were replaced for the mouse cross-reactive ones of B20-4.1)). The antibody was prepared as described in WO2011/117329. Based on the mechanism of action similar results are expected in human patient with the bispecific ANG2/VEGF antibody ((SEQ ID NOs:7-10) which is specific for human VEGF (but has no mouse VEGF crossreactivity). The bispecific HER2 antibody is a bispecific HER2 antibody with a common light chain including the two heavy chain variable domains of SEQ ID NOs: 11-12, and two common light chain variable domains of SEQ ID NO: 13. The antibody was prepared as described in WO2015/091738 (comprising the two heavy chains of SEQ ID NOs: 14-15 and two identical common light chains of SEQ ID NO: 16.

Cell line KPL4

This human breast cancer cell line has been established from the malignant pleural effusion of a breast cancer patient with an inflammatory skin metastasis. Cells have been provided by Professor J. Kurebayashi (Kawasaki Medical School, Kurashiki, Japan). Tumor cells are routinely cultured in DMEM medium (PAN Biotech, Germany) supplemented with 10 % fetal bovine serum (PAN Biotech, Germany) and 2 mM L-glutamine (PAN Biotech, Germany) at 37 °C in a water-saturated atmosphere at 5 % CO₂. Culture passage is performed with trypsin / EDTA 1x (PAN) splitting twice / week. Cell passage P6 is used for *in vivo* study.

Mice

Female SCID beige (C.B.-17) mice; age 10-12 weeks; body weight 18-20 g (Charles River Germany, Sulzfeld); body weight >20 g are maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to international guidelines (GV-Solas; Felasa; TierschG). After arrival animals are housed in the quarantine part of the animal facility for one week to get accustomed to new environment and for observation. Continuous health monitoring is carried out on regular basis. Diet food (Alltromin) and water are provided ad libitum. The experimental study was reviewed and approved by local government.

Tumor cell injection

At the day of injection tumor cells are harvested (trypsin-EDTA) from culture flasks (Greiner TriFlask) and transferred into 50 ml culture medium, washed once and resuspended in PBS. After an additional washing step with PBS and filtration (cell strainer; Falcon Ø 100µm) the final cell titer is adjusted to 1.5 x 10⁸ / ml. Tumor cell suspension is carefully mixed with transfer pipette to avoid cell aggregation. Anesthesia is performed using a Stephens inhalation unit for small animals with preincubation chamber (plexiglas), individual mouse nose-mask (silicon) and not flammable or explosive anesthesia compound Isoflurane (Pharmacia-Upjohn, Germany) in a closed circulation system. Two days before injection, coat of the SCID beige mice is shaved and KPL-4 cells (3 x 10⁶ cells) are injected orthotopically in a volume of 20 µl (using a Hamilton microliter syringe and a 30Gx1/2" needle) into the right penultimate inguinal mammary fat pad of each anesthetized mouse. The cell suspension is injected through the skin under the nipple using.

Monitoring

Animals are controlled daily for detection of clinical symptoms of adverse effects. For monitoring throughout the experiment the body weight of the animals is documented two times weekly and the tumor volume is measured by caliper twice weekly. Tumor volume was calculated according to NCI protocol (Tumor weight = $1/2ab^2$, where “a” and “b” are the long and the short diameters of the tumor, respectively). Termination criteria were the critical tumor mass (up to 1.7 g or $\varnothing > 1.5$ cm), body weight loss more than 20% from baseline, tumor ulceration or poor general condition of the animals. Study exclusion criteria for the animals are described and approved in the corresponding “Tierversuchsanzeige”.

Treatment

Mice were randomized for tumor volume of 85mm³ and subsequently treated once weekly for 4 weeks with a dosage of 3 mg/kg. For combination treatment the bispecific anti-HER2 antibody was given first and the bispecific Ang2/VEGF antibody was given 24 hrs thereafter.

Results of the in vivo anti-tumor efficacy of bispecific anti-ANG2/VEGF antibody in combination with bispecific anti-HER2 domainII/domainIV antibody are shown in Figure 1 compared to the efficacy of control and the single agents

Regarding tumor volume measurements, the combination of both bispecific antibodies at a dosage of 3 mg/kg dosage showed synergistic effects and revealed a significant inhibition of tumor growth.

Patent Claims

1. A bispecific anti-ANG2/VEGF comprising a first antigen binding site specific for ANG2 and a second antigen binding site specific for the VEGF; wherein the antibody is administered in combination with a bispecific anti-HER2 antibody comprising a first antigen binding site specific for the domain II of HER2 and a second antigen binding site specific for the domain IV of HER2
 - a) for use in treating cancer or delaying progression of cancer, or
 - b) for use in prolonging the survival of a patient suffering from cancer.
2. The antibody for use according to claim 1, wherein the bispecific anti-ANG2/VEGF antibody is monovalent for both ANG2 and VEGF, wherein the bispecific anti-HER2 antibody is monovalent for both the domain II and IV of HER2.
3. The antibody for use according to any one of the preceding claims, wherein the bispecific anti-ANG2/VEGF antibody and the bispecific anti-HER2 antibody are both IgG antibodies.
4. The antibody for use according to any one of the preceding claims
 - i) wherein the bispecific anti-ANG2/VEGF antibody comprises a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:1 and a light chain variable comprising domain amino acid sequence of SEQ ID NO:2; and a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:5 and a light chain variable domain comprising amino acid sequence of SEQ ID NO:6;
 - and
 - ii) wherein the bispecific anti-HER2 antibody comprises a heavy chain variable domain comprising amino acid sequence of SEQ ID NO: 11; a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:12, and two light chain variable domains comprising amino acid sequence of SEQ ID NO: 13.

5. The antibody for use according to any one of the preceding claims
- i) wherein the bispecific anti-ANG2/VEGF antibody comprises
a heavy chain comprising amino acid sequence SEQ ID NO: 7;
a heavy chain comprising amino acid sequence SEQ ID NO: 8;
5 a light chain comprising amino acid sequence SEQ ID NO: 9; and
a light chain comprising amino acid sequence SEQ ID NO: 10;
and
- ii) wherein the bispecific anti-HER2 antibody comprises
a heavy chain comprising amino acid sequence SEQ ID NO: 14;
10 a heavy chain comprising amino acid sequence SEQ ID NO: 15; and
two light chains comprising amino acid sequence SEQ ID NO: 16.
6. The antibody for use according to any one of the preceding claims, wherein
the cancer is lung cancer, non small cell lung (NSCL) cancer,
bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin
15 cancer, cancer of the head or neck, cutaneous or intraocular melanoma,
uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region,
stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer,
carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma
of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's
20 Disease, cancer of the esophagus, cancer of the small intestine, cancer of the
endocrine system, cancer of the thyroid gland, cancer of the parathyroid
gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the
urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of
the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis,
25 mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central
nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma
multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas,
meningiomas, squamous cell carcinomas, pituitary adenoma, lymphoma or
lymphocytic leukemia.
7. The antibody for use according to claim 6, wherein the cancer is a solid
30 tumor.

8. The antibody for use according to claims 6 or 7, wherein the cancer is further characterized by HER2 expression (in embodiment by HER2 overexpression).
- 5 9. The antibody for use according to any one of embodiments 6 to 8, wherein the cancer is colon cancer, ovarian cancer, glioblastoma, gastric cancer, pancreatic cancer, breast cancer, lung cancer, hepatocellular cancer.
- 10 10. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered simultaneously.
- 11 11. The antibody for use according to any one of the preceding embodiments, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are administered sequentially.
12. A pharmaceutical kit comprising:
 - 15 a bispecific anti-ANG2/VEGF antibody comprising a first antigen binding site specific for ANG2 and a second antigen binding site specific for the VEGF;
 - and a bispecific anti-HER2 antibody comprising a first antigen binding site specific for the domain II of HER2 and a second antigen binding site specific for the domain IV of HER2
 - 20 wherein the antibodies are to be administered in combination a) for use in treating cancer, or b) for use in delaying progression of cancer, or c) for use in prolonging the survival of a patient suffering from cancer.
- 25 13. The kit according to claim 12, wherein the bispecific anti-ANG2/VEGF antibody is monovalent for both ANG2 and VEGF, wherein the bispecific anti-HER2 antibody is monovalent for both the domain II and IV of HER2.
14. The kit according to any one of the preceding claims, wherein the bispecific anti-ANG2/VEGF antibody and the bispecific anti-HER2 antibody are both IgG antibodies.
15. The kit according to any one of the preceding claims
 - 30 i) wherein the bispecific anti-ANG2/VEGF antibody comprises a heavy chain variable domain comprising amino acid sequence of SEQ ID

NO:1 and a light chain variable comprising domain amino acid sequence of SEQ ID NO:2; and

a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:5 and a light chain variable domain comprising amino acid sequence of SEQ ID NO:6;

and

ii) wherein the bispecific anti-HER2 antibody comprises

a heavy chain variable domain comprising amino acid sequence of SEQ ID NO: 11;

a heavy chain variable domain comprising amino acid sequence of SEQ ID NO:12, and

two light chain variable domains comprising amino acid sequence of SEQ ID NO: 13.

16. The kit according to any one of the preceding claims

i) wherein the bispecific anti-ANG2/VEGF antibody comprises

a heavy chain comprising amino acid sequence SEQ ID NO: 7;

a heavy chain comprising amino acid sequence SEQ ID NO: 8;

a light chain comprising amino acid sequence SEQ ID NO: 9; and

a light chain comprising amino acid sequence SEQ ID NO: 10;

and

ii) wherein the bispecific anti-HER2 antibody comprises

a heavy chain comprising amino acid sequence SEQ ID NO: 14;

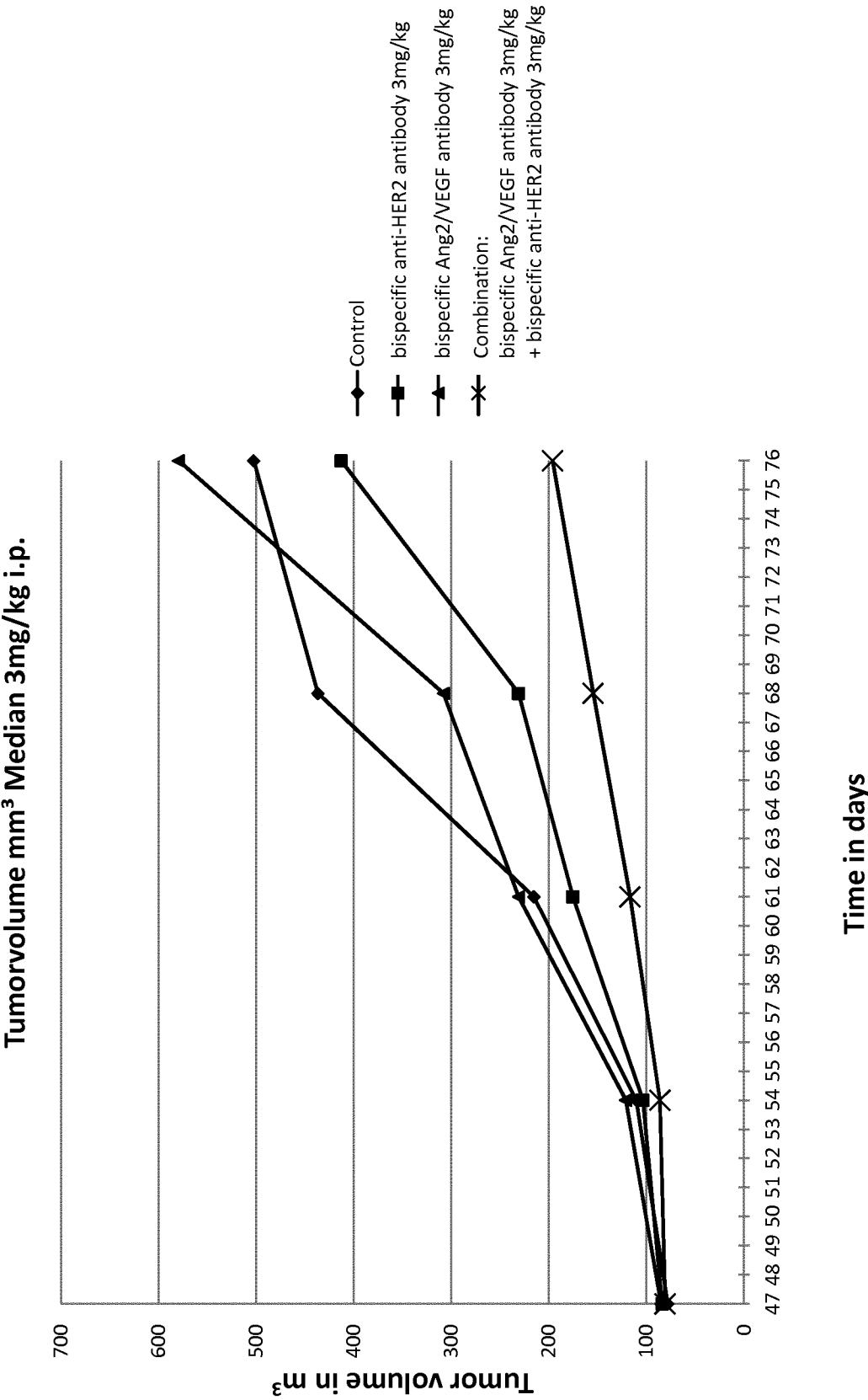
a heavy chain comprising amino acid sequence SEQ ID NO: 15; and

two light chains comprising amino acid sequence SEQ ID NO: 16.

17. The kit according to any one of the preceding claims, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are to be administered simultaneously.

18. The kit according to any one of the preceding claims, wherein the bispecific anti-ANG2/VEGF and the bispecific anti-HER2 antibody are to be administered sequentially.

Figure 1



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/083187

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/22 C07K16/32 A61P35/00 A61K39/395
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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Date of the actual completion of the international search

12 February 2018

Date of mailing of the international search report

19/02/2018

Name and mailing address of the ISA/

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Authorized officer

Siaterli, Maria

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/083187

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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