Anti-HER3 antibodies and uses thereof

The present invention relates to antibodies binding to human HER3 (anti-HER3 antibody), methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

Background of the Invention

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

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

WO 97/35885 relates to HER3 antibodies. WO 2003/013602 relates to inhibitors of HER activity, including HER antibodies. WO 2007/077028 and WO 2008/100624 also relate to HER3 antibodies.

Summary

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

The disclosure further comprises an antibody according to the disclosure characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9, or the light chain variable domain VL is SEQ ID NO:10, or the light chain variable domain VL is SEQ ID NO:11; or a humanized version thereof. Hence, in a first aspect, there is provided an antibody which is binding to human HER3, characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9, or the light chain variable domain VL is SEQ ID NO:10, or the light chain variable domain VL is SEQ ID NO:11; or a humanized version thereof.

The disclosure further comprises an antibody comprising a heavy chain variable domain comprising a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and a light chain variable domain comprising a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6.

The disclosure further comprises an antibody comprising a heavy chain variable domain VH of SEQ ID NO:8; and the light chain variable domain VL of SEQ ID NO:9 or SEQ ID NO:11.

The disclosure further comprises an antibody comprising a heavy chain variable domain comprising a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprising a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:7.

The disclosure further comprises an antibody comprising a heavy chain variable domain VH of SEQ ID NO:8; and a light chain variable domain VL of SEQ ID NO:10.

The disclosure also provides for an antibody which binds to human HER3, where the antibody comprises a heavy chain variable domain VH having at least 95% sequence identity to SEQ ID NO:8 and a light chain variable domain VL having at least 95% sequence identity to SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

In one embodiment the antibody according to the disclosure is characterized in that the antibody is monoclonal.

In one embodiment the antibody according to the disclosure is characterized in that the antibody is humanized.

In one embodiment the antibody according to the disclosure is characterized in that the antibody is of IgG1, or IgG4 subclass

In a second aspect, there is provided a pharmaceutical composition characterized by comprising an antibody according to the disclosure.

In a third aspect, there is provided an antibody according to the disclosure for the treatment of cancer.

In a fourth aspect, there is provided the use of an antibody according to the disclosure for the manufacture of a medicament for the treatment of cancer.

The disclosure further comprises a method for the treatment of a patient suffering from cancer characterized by administering to the patient an antibody according to the invention.

The disclosure also provides for a nucleic acid encoding a heavy and a light chain of an anti-HER3 antibody provided herein. In a fifth aspect, there is provided a nucleic acid encoding a heavy and light chain of an antibody binding to human HER3, characterized in that said antibody comprises a variable domain as defined above. In one embodiment, the antibody comprises a heavy chain variable domain VH having at least 95% sequence identity to SEQ ID NO:8 and a light chain variable domain VL having at least 95% sequence identity to SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

The disclosure further comprises a nucleic acid encoding a heavy and a light chain of an antibody binding to human HER3, characterized in that said antibody comprises a variable domain VH of SEQ ID NO:8; and a light chain variable

domain VL of SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:11; or a humanized version thereof.

In a sixth aspect, there is provided an expression vector characterized in comprising a nucleic acid according to the disclosure for the expression of the antibody according to the disclosure in a prokaryotic or eukaryotic host cell.

In a seventh aspect, there is provided a prokaryotic or eukaryotic host cell comprising a vector according to the disclosure.

In an eighth aspect, there is provided a method for the production of a recombinant antibody according to the disclosure, characterized by expressing a nucleic acid according to the disclosure in a prokaryotic or eukaryotic host cell and recovering said antibody from said cell or the cell culture supernatant.

Surprisingly it was found that the antibodies according to the disclosure have highly valuable properties such as strong growth inhibition of HER3 expressing cancer cells, strong inhibition of HER3 mediated signal transduction (such as e.g HER3 phoshorylation and AKT phosporylation) which is related to cancer cell proliferation, high binding affinity to HER3, or excellent pharmacokinetic properties (such as long half time, etc.).

Detailed Description of the Invention

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

The invention further comprises an antibody according to the invention characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9, or the light chain variable domain VL is SEQ ID NO:10, or the light chain variable domain VL is SEQ ID NO:11; or a humanized version thereof.

The invention further comprises an antibody according to the invention characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9, or the light chain variable domain VL is SEQ ID NO:10, or the light chain variable domain VL is SEQ ID NO:11.

In one embodiment the antibody according to the invention is characterized in comprising as heavy chain variable domain a CDR3H region of SEQ ID NO: 1, a CDR2H region of SEQ ID NO: 2, and a CDR1H region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3L region of SEQ ID NO: 4, a CDR2L region of SEQ ID NO:5, and a CDR1L region of SEQ ID NO:6.

In one embodiment the antibody according to the invention is characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9 or the light chain variable domain VL is SEQ ID NO:11.

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

In one embodiment the antibody according to the invention is characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:10.

In one embodiment the antibody the according to the invention is monoclonal. In one embodiment the antibody according to the invention is humanized or human. In one embodiment the antibody according to the invention is of IgG1 or IgG4 subclass. In one embodiment the antibody according to the invention is a monoclonal humanized antibody of IgG1 subclass. In one embodiment the antibody according to the invention is characterized in that said antibody is glycosylated with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65 % or lower.

The invention comprises the humanized antibodies Mab 205.10.1, Mab 205.10.2 and Mab 205.10.3 with their respective VH and VL or CDRs.

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

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

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

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

"Antibody fragments" comprise a portion of a full length antibody, preferably the variable domain thereof, or at least the antigen binding site thereof. Examples of antibody fragments include diabodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. scFv antibodies are, e.g., described in Huston, J.S., Methods in Enzymol. 203 (1991) 46-88. In addition, antibody fragments comprise single chain polypeptides having the characteristics of a V_H domain, namely being able to assemble together with a V_L domain, or of a V_L domain binding to HER3, namely being able to assemble together with a V_H domain to a functional antigen binding site and thereby providing the properties of an antibody according to the invention.

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

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

The term "humanized antibody" or "humanized version of an antibody" refers to antibodies in which the framework or "complementarity determining regions" (CDR) have been modified to comprise the CDR of an immunoglobulin of different specificity as compared to that of the parent immunoglobulin. In a preferred embodiment, the CDRs of the VH and VL are grafted into the framework region of human antibody to prepare the "humanized antibody." See e.g. Riechmann, L., et al., Nature 332 (1988) 323-327; and Neuberger, M.S., et al., Nature 314 (1985) 268-270. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies. Human heavy and light chain variable framework regions are listed e.g. in Lefranc, M.-P., Current Protocols in Immunology (2000) - Appendix 1P A.1P.1-A.1P.37 and are accessible via IMGT, the international ImMunoGeneTics information system® (http://imgt.cines.fr) or via http://vbase.mrc-cpe.cam.ac.uk. Optionally the framework region can be modified by further mutations. Particularly preferred CDRs correspond to those representing sequences recognizing the antigens noted above for chimeric antibodies. Preferably such humanized version is chimerized with a human constant region (see e.g. Sequences SEQ ID NO:12-16). The term "humanized antibody" as used herein also comprises such antibodies which are modified in the constant region to generate the properties according to the

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

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germ line immunoglobulin sequences. Human antibodies are well-known in the state of the art (van Dijk, M.A., and van de Winkel, J.G., Curr. Opin. Chem. Biol. 5 (2001) 368-374). Human antibodies can also be produced in transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire or a selection of human antibodies in the absence of endogenous immunoglobulin production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits, A., et al., Proc. Natl. Acad. Sci. USA 90 (1993) 2551-2555; Jakobovits, A., et al., Nature 362 (1993) 255-258; Brueggemann, M.D., et al., Year Immunol. 7 (1993) 33-40). Human antibodies can also be produced in phage display libraries (Hoogenboom, H.R., and Winter, G., J. Mol. Biol. 227 (1992) 381-388; Marks, J.D., et al., J. Mol. Biol. 222 (1991) 581-597). The techniques of Cole, A., et al. and Boerner, P., et al. are also available for the preparation of human monoclonal antibodies (Cole, A., et al., Monoclonal Antibodies and Cancer Therapy, Liss, A.L., p. 77 (1985); and Boerner, P., et al., J. Immunol. 147 (1991) 86-95). As already mentioned for and humanized antibodies according to the invention the term "human antibody" as used herein also comprises such antibodies which are modified in the constant region to generate the properties according to the invention, especially in regard to C1q binding and/or FcR binding, e.g. by "class switching" i.e. change or mutation of Fc parts (e.g. from IgG1 to IgG4 and/or IgG1/IgG4 mutation.)

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from a host cell such as a NS0 or CHO cell or from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes or antibodies expressed using a recombinant expression vector transfected into a host cell. Such recombinant human antibodies have variable and constant regions in a rearranged form. The recombinant human antibodies according to the invention have been subjected to in vivo somatic hypermutation. Thus, the amino acid

sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germ line VH and VL sequences, may not naturally exist within the human antibody germ line repertoire in vivo.

As used herein, the terms "binding to human HER3", "specifically binding to human HER3", or " anti-HER3 antibody" are interchangeable and refer to an antibody specifically binding to the human HER3 antigen with a binding affinity of KD-value of 1.0 x 10^{-8} mol/l or lower at 25°C, in one embodiment of a KD-value of 1.0 x10^{-9} mol/l or lower at 25°C. The binding affinity is determined with a standard binding assay at 25°C, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden). A method for determining the KD-value of the binding affinity is described in Example 2b). Thus an "antibody binding to human HER3" as used herein refers to an antibody specifically binding to the human HER3 antigen with a binding affinity of KD 1.0 x 10^{-8} mol/l or lower (preferably 1.0 x 10^{-8} mol/l - 1.0 x 10^{-12} mol/l) at 25°C.

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

Chem. 269 (1994) 14661-14665; Alimandi, M., et al, Oncogene 10 (1995) 1813-1821; Hellyer, N.J., J. Biol. Chem. 276 (2001) 42153-421561; Singer, E., J. Biol. Chem. 276 (2001) 44266-44274; Schaefer, K.L., Neoplasia 8 (2006) 613-622).

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

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

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

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

The term "antigen-binding portion of an antibody" when used herein refer to the amino acid residues of an antibody which are responsible for antigen-binding. The

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

The "Fc part" of an antibody is not involved directly in binding of an antibody to an antigen, but exhibit various effector functions. A "Fc part of an antibody" is a term well known to the skilled artisan and defined on the basis of papain cleavage of antibodies. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies or immunoglobulins are divided in the classes: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG1, IgG2, IgG3, and IgG4, IgA1, and IgA2. According to the heavy chain constant regions the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The Fc part of an antibody is directly involved in ADCC (antibody-dependent cell-mediated cytotoxicity) and CDC (complement-dependent cytotoxicity) based on complement activation, C1q binding and Fc receptor binding. The term "complement-dependent cytotoxicity (CDC)" denotes a process initiated by binding of complement factor C1q to the Fc part of most IgG antibody subclasses. Binding of C1q to an antibody is caused by defined protein-protein interactions at the so called binding site. Such binding sites are known in the state of the art and described e.g. by Boackle, R.J., et al., Nature 282 (1979) 742-743, Lukas, T.J., et al., J. Immunol. 127 (1981) 2555-2560, Brunhouse, R., and Cebra, J.J., Mol. Immunol. 16 (1979) 907-917, Burton, D.R., et al., Nature 288 (1980) 338-344, Thommesen, J.E., et al., Mol. Immunol. 37 (2000) 995-1004, Idusogie, E.E., et al., J. Immunol.164 (2000) 4178-4184, Hezareh, M., et al., J. Virology 75 (2001) 12161-12168, Morgan, A., et al., Immunology 86 (1995) 319-

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

In one embodiment the antibody according to the invention comprises a Fc part derived from human origin and preferably all other parts of the human constant regions. As used herein the term "Fc part derived from human origin" denotes a Fc part which is either a Fc part of a human antibody of the subclass IgG1, IgG2, IgG3 or IgG4, e.g. a Fc part from human IgG1 subclass, a mutated Fc part from human IgG1 subclass (preferably with a mutation on L234A + L235A), a Fc part from human IgG4 subclass or a mutated Fc part from human IgG4 subclass (preferably with a mutation on S228P). Preferred are the human heavy chain constant regions of SEQ ID NO: 13 (human IgG1 subclass), SEQ ID NO: 14 (human IgG1 subclass with mutations L234A and L235A).

In one embodiment the antibody according to the invention is of human IgG1 subclass or of human IgG3 subclass. In one embodiment the antibody according to the invention is of human IgG1 subclass.

In one embodiment the antibody according to the invention 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). For example, a useful human heavy chain constant region comprises an amino acid sequence of SEQ ID NO: 13. For example, a useful human light chain constant region comprises an amino acid sequence of a kappa-light chain constant region of SEQ ID NO: 12.

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

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

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

A further embodiment of the invention is a nucleic acid encoding a heavy and a light chain of an antibody according to the invention.

The antibodies according to the invention include, in addition, such antibodies having "conservative sequence modifications" (variant antibodies), nucleotide and amino acid sequence modifications which do not affect or alter the above-mentioned characteristics of the antibody according to the invention. Modifications can be introduced by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g. lysine, arginine, histidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g. alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g. threonine, valine, isoleucine) and aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a human anti-HER3 antibody can be preferably replaced with another amino acid residue from the same side chain family. A "variant" anti-HER3 antibody, refers therefore herein to a molecule which differs in amino acid sequence from a "parent" anti-HER3 antibody amino acid sequence by up to ten, preferably from about two to about five, additions, deletions and/or substitutions in one or more variable region of the parent antibody. Amino acid substitutions can be performed by mutagenesis based upon molecular modeling as described by Riechmann, L., et al., Nature 332 (1988) 323-327 and Queen, C., et al., Proc. Natl. Acad. Sci. USA 86 (1989) 10029-10033.

In another aspect, an anti-HER3 antibody according to the invention comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:8. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti HER3 antibody comprising that sequence retains the ability to bind to HER3. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:8. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). Optionally, the anti-HER3 antibody comprises the VH

sequence in SEQ ID NO:8, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three CDRs selected from: (a) CDR1H comprising the amino acid sequence of SEQ ID NO:3, (b) CDR2H comprising the amino acid sequence of SEQ ID NO:2, and (c) CDR3H comprising the amino acid sequence of SEQ ID NO:1.

In another aspect, an anti-HER3 antibody according to the invention comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-HER antibody comprising that sequence retains the ability to bind to HER. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). Optionally, the anti-HER3 antibody comprises the VL sequence in SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three CDRs selected from (a) CDR1L comprising the amino acid sequence of SEQ ID NO:6, or SEQ ID NO:7; (b) CDR2L comprising the amino acid sequence of SEQ ID NO:5; and (c) CDR3L comprising the amino acid sequence of SEQ ID NO:4.

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

- the anti-HER3 antibody inhibits the HER3 phosphorylation in tumor cells such as MCF7 cells, FaDu cells or Mel-Juso cell (in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in MCF7 cells of at least 80% (in one embodiment at least 90%) at a

concentration of 1.0 µg/ml ; in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in FaDu cells of at least 80% (in one embodiment at least 90%) at a concentration of 0.1 µg/ml ; in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in Mel-Juso cells of at least 60% (in one embodiment at least 70%) at a concentration of 0.1 µg/ml)

- the anti-HER3 antibody inhibits the AKT phosphorylation in tumor cells such as Mel-Juso cell (in one embodiment the anti-HER3 antibody inhibits the AKT phosphorylation in Mel-Juso cells with an IC50 value of less than 0.50 µg/ml, in one embodiment with IC50 value of less than 0.35 µg/ml)

- the anti-HER3 antibody inhibits the proliferation of tumor cells such as MDA-MB-175 cells (in one embodiment the anti-HER3 antibody inhibits the proliferation of MDA-MB-175 cells with an IC50 value of less than 10 µg/ml)

- the anti-HER3 antibody binds to HER3 with a KD value of less than $5.0x\ 10^{-9}$ M, in one embodiment with a KD value of less than $3.0x\ 10^{-9}$ M.

- In another aspect, an anti-HER3 antibody according to the inventions comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:8 and comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11; and has one or more of the following properties (determined in assays as described in Example 3 and 2):the anti-HER3 antibody inhibits the HER3 phosphorylation in tumor cells such as MCF7 cells, FaDu cells or Mel-Juso cell (in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in MCF7 cells of at least 80% (in one embodiment at least 90%) at a concentration of 1.0 µg/ml ; in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in FaDu cells of at least 80% (in one embodiment at least 90%) at a concentration of 0.1 µg/ml ; in on embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in Mel-Juso

cells of at least 60% (in one embodiment at least 70%) at a concentration of 0.1 μg/ml)

- the anti-HER3 antibody inhibits the AKT phosphorylation in tumor cells such as Mel-Juso cell (in one embodiment the anti-HER3 antibody inhibits the AKT phosphorylation in Mel-Juso cells with an IC50 value of less than 0.50 μg/ml, in one embodiment with IC50 value of at least 0.35 μg/ml)

- the anti-HER3 antibody inhibits the proliferation of tumor cells such as MDA-MB-175 cells (in on embodiment the anti-HER3 antibody inhibits the proliferation of MDA-MB-175 cells with an IC50 value of less than 10 μg/ml)

- the anti-HER3 antibody binds to HER3 with a KD value of less than 5.0×10^{-9} M, in one embodiment with a KD value of less than 3.0×10^{-9} M.

One embodiment of the invention is an antibody which is binding to human HER3, comprising a heavy chain variable domain VH having at least 95% sequence identity to SEQ ID NO:8 and a light chain variable domain VL having at least 95% sequence identity to SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11.

One embodiment of the invention is such antibody, wherein the light chain variable domain VL has at least 95% sequence identity to SEQ ID NO:9.

One embodiment of the invention is such antibody, wherein the light chain variable domain VL has at least 95% sequence identity to SEQ ID NO:10.

One embodiment of the invention is such antibody, wherein the light chain variable domain VL has at least 95% sequence identity to SEQ ID NO:11.

One embodiment of the invention is an antibody which is binding to human HER3, comprising a heavy chain variable domain VH having at least 95% sequence identity to SEQ ID NO:8 and a light chain variable domain VL having at least 95% sequence identity to SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11; and has one or more of the following properties (determined in assays as described in Example 3 and 2):

- the anti-HER3 antibody inhibits the HER3 phosphorylation in tumor cells such as MCF7 cells, FaDu cells or Mel-Juso cell (in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in MCF7 cells of at least 80% (in one embodiment at least 90%) at a concentration of 1.0 µg/ml ; in one embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in FaDu cells of at least 80% (in one embodiment at least 90%) at a concentration of 0.1 µg/ml ; in on embodiment the anti-HER3 antibody shows an inhibition of the HER3 phosphorylation in Mel-Juso cells of at least 60% (in one embodiment at least 70%) at a concentration of 0.1 µg/ml)

- the anti-HER3 antibody inhibits the AKT phosphorylation in tumor cells such as Mel-Juso cell (in one embodiment the anti-HER3 antibody inhibits the AKT phosphorylation in Mel-Juso cells with an IC50 value of less than 0.50 µg/ml, in one embodiment with IC50 value of at least 0.35 µg/ml)

- the anti-HER3 antibody inhibits the proliferation of tumor cells such as MDA-MB-175 cells (in on embodiment the anti-HER3 antibody inhibits the proliferation of MDA-MB-175 cells with an IC50 value of less than 10 µg/ml)

- the anti-HER3 antibody binds to HER3 with a KD value of less than 5.0×10^{-9} M, in one embodiment with a KD value of less than 3.0×10^{-9} M.

Identity or homology with respect to the sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the parent sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence shall be construed as affecting sequence identity or homology. The variant retains the ability to bind the variable domain of human HER3 and preferably has properties, which are superior to those of the parent antibody. For example, the variant may have reduced side effects during treatment.

An exemplary "parent" antibody comprises the CDR regions of antibody Mab 205.10.2 and is preferably used for the preparation of the variant. Preferably, the parent antibody has a human framework region and, if present, has human antibody

constant domains. For example, the parent antibody may be a humanized or a human antibody.

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

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

Methods to enhance cell-mediated effector functions of monoclonal antibodies via glycoengineering are reported e.g. in WO 2005/044859, WO 2004/065540,

WO2007/031875, Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180, WO 99/154342, WO 2005/018572, WO 2006/116260, WO 2006/114700, WO 2004/065540, WO 2005/011735, WO 2005/027966, WO 1997/028267, US 2006/0134709, US 2005/0054048, US 2005/0152894, WO 2003/035835 and WO 2000/061739 or e.g. in Niwa, R., et al., J. Immunol. Methods 306 (2005) 151-160; Shinkawa, T., et al, J Biol Chem, 278 (2003) 3466–3473; WO 03/055993 and US 2005/0249722.

In one embodiment of the invention, the antibody according to the invention is afucosylated wich means the antibody is glycosylated (if it comprises an Fc part of IgG1 or IgG3 subclass) with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 80% or lower (Numbering according to Kabat), e.g. between 80% and 1 %. In another embodiment is the amount of fucose within said sugar chain is 65% or lower, in one embodiment between 5% and 65%, and in one embodiment the amount of fucose within said sugar chain is 0%. Such antibodies are referred to in the following as "afucosylated antibodies" or "non-fucosylated antibodies". Such afucosylated antibodies show enhanced ADCC whereas other antibody properties remain substantially unaffected.

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

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

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

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

Glycosylation of human IgG1 or IgG3 occurs at Asn297 as core fucosylated biantennary complex oligosaccharide glycosylation terminated with up to two Gal residues. Human constant heavy chain regions of the IgG1 or IgG3 subclass are reported in detail by Kabat, E., A., et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991), and by Brueggemann, M., et al., J. Exp. Med. 166 (1987) 1351-1361; Love, T.W., et al., Methods Enzymol. 178 (1989) 515-527. These structures are designated as G0, G1 (α-1,6- or α-1,3-), or G2 glycan residues, depending from the amount of terminal Gal residues (Raju, T.S., Bioprocess Int. 1 (2003) 44-53). CHO type glycosylation of antibody Fc parts is e.g. described by Routier, F.H., Glycoconjugate J. 14 (1997) 201-207. Antibodies which are recombinantly expressed in non-glycomodified CHO host cells usually are fucosylated at Asn297 in an amount of at least 85%. The modified oligosaccharides of the full length parent antibody may be hybrid or complex. Preferably the bisected, reduced/not-fucosylated oligosaccharides are hybrid. In another embodiment, the bisected, reduced/not-fucosylated oligosaccharides are complex.

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

The antibodies according to the invention are preferably produced by recombinant means. Such methods are widely known in the state of the art and comprise protein expression in prokaryotic and eukaryotic cells with subsequent isolation of the antibody polypeptide and usually purification to a pharmaceutically acceptable purity. For the protein expression nucleic acids encoding light and heavy chains or fragments thereof are inserted into expression vectors by standard methods. Expression is performed in appropriate prokaryotic or eukaryotic host cells, such as

CHO cells, NS0 cells, SP2/0 cells, HEK293 cells, COS cells, yeast, or E. coli cells, and the antibody is recovered from the cells (from the supernatant or after cells lysis). Recombinant production of antibodies is well-known in the state of the art and described, for example, in the review articles of Makrides, S.C., Protein Expr. Purif. 17 (1999) 183-202; Geisse, S., et al., Protein Expr. Purif. 8 (1996) 271-282; Kaufman, R.J., Mol. Biotechnol. 16 (2000) 151-161; Werner, R.G., Drug Res. 48 (1998) 870-880. The antibodies may be present in whole cells, in a cell lysate, or in a partially purified, or substantially pure form. Purification is performed in order to eliminate other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by standard techniques, including, column chromatography and others well known in the art (see Ausubel, F., et al., ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987)). Expression in NS0 cells is described by, e.g., 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. 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 is readily isolated and sequenced using conventional procedures. The hybridoma cells can serve as a source of such DNA and RNA. Once isolated, the DNA may be inserted into expression vectors, which are then transfected into host cells, such as HEK 293 cells, CHO cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of recombinant monoclonal antibodies in the host cells. Antibodies obtainable from said cell lines are preferred embodiments of the invention. Afocusylated antibodies are preferably prepared via glycoengineering as descrined above.

Amino acid sequence variants of anti-HER3 antibody are prepared by introducing appropriate nucleotide changes into the antibody encoding DNA, or by peptide synthesis. Such modifications can be performed, however, only in a very limited range, e.g. as described above. For example, the modifications do not alter the abovementioned antibody characteristics such as the IgG isotype and epitope binding, but may improve the yield of the recombinant production, protein stability, or facilitate the purification. Any cysteine residue not involved in maintaining the proper conformation of the anti-HER3, antibody may also be substituted, generally with serine, to improve the oxidative stability of the molecule and to prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment). Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By "altering" is meant removing one or more carbohydrate moieties found in the antibody and/or adding one or more glycosylation sites that are not present in the antibody. Glycosylation of antibodies is typically N-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites).

Nucleic acid molecules encoding amino acid sequence variants of anti-HER3 antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of humanized anti- HER3 antibody.

Another type of covalent modification of the antibody comprises linking the antibody to one of a variety of non proteinaceous polymers, e.g., polyethylene

glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in US Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; 4,179,337.

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.

One aspect of the invention is a pharmaceutical composition comprising an antibody according to the invention. Another aspect of the invention is the use of an antibody according to the invention for the manufacture of a pharmaceutical composition. A further aspect of the invention is a method for the manufacture of a pharmaceutical composition comprising an antibody according to the invention. In another aspect, the present invention provides a composition, e.g. a pharmaceutical composition, containing an antibody according to the present invention, formulated together with a pharmaceutical carrier.

Furthermore the anti-HER3 antibodies according to the invention have turned out to be especially useful for the treatment of cancer.

Therefore one aspect of the invention is said pharmaceutical composition for the treatment of cancer.

Another aspect of the invention is an antibody according to the invention for the treatment of cancer.

Another aspect of the invention is the use of an antibody according to the invention for the manufacture of a medicament for the treatment of cancer.

Another aspect of the invention is a method of treatment of a patient suffering from cancer by administering an antibody according to the invention to said patient in the need of such treatment.

As used herein, "pharmaceutical carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption

delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g. by injection or infusion).

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

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

The term "cancer" as used herein may be, for example, 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, schwanomas, ependymonas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma, lymphoma, lymphocytic leukemia, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers. Preferably such cancer is a breast cancer, lung cancer, cancer of the head or neck, or pancreatic cancer, preferably lung cancer, cancer of the head or neck, or pancreatic cancer . Preferably such cancers are further characterized by HER3 expression or overexpression, more preferably by HER3 overexpression.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

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.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with

the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

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

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

The following examples, sequence listing and figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Description of the Sequence Listing

SEQ ID NO: 1	heavy chain CDR3H, Mab 205.10
SEQ ID NO: 2	heavy chain CDR2H, Mab 205.10
SEQ ID NO: 3	heavy chain CDR1H, Mab 205.10
SEQ ID NO: 4	light chain CDR3L, Mab 205.10
SEQ ID NO: 5	light chain CDR2L, Mab 205.10
SEQ ID NO: 6	light chain CDR1L (variant 1), Mab 205.10
SEQ ID NO: 7	light chain CDR1L (variant 2), Mab 205.10
SEQ ID NO: 8	heavy chain variable domain VH, Mab 205.10
SEQ ID NO: 9	light chain variable domain VL, Mab 205.10.1
SEQ ID NO: 10	light chain variable domain VL, Mab 205.10.2
SEQ ID NO: 11	light chain variable domain VL, Mab 205.10.3
SEQ ID NO: 12	human kappa light chain constant region
SEQ ID NO: 13	human heavy chain constant region derived from IgG1
SEQ ID NO: 14	human heavy chain constant region derived from IgG1 mutated on L234A and L235A
SEQ ID NO: 15	human heavy chain constant region derived from IgG4

SEQ ID NO: 16	human heavy chain constant region derived from IgG4 mutated on S228P
SEQ ID NO: 17	human HER3

Description of the Figures

Figure 1A and B:	Percent (%) inhibition of anti-HER3 antibodies on receptor phosphorylation in MCF7 cells in different concentrations
Figure 1C	Percent (%) inhibition of anti-HER3 antibodies on receptor phosphorylation in Mel-Juso cells in different concentrations
Figure 2	Treatment with Mab 205 (10mg/kg q7dx3, i.p.) resulted in tumor stasis of FaDu SCCHN transplanted xenografts
Figure 3	Treatment with Mab 205 (10mg/kg q7d, i.p.) resulted in tumor stasis of MAXF449 breast cancer transplanted xenografts
Figure 4	Treatment with Mab 205 (25mg/kg q7d, i.p.) resulted in tumor stasis of 7177 NSCLC transplanted xenografts

Examples

Example 1

Immunisation

NMRI mice were immunized with hHER3-ECD (inhouse) and boosted with hu-HER3-ECD. The immune response was monitored by testing serum samples against the HER1/2/3- ECD-ELISA. Spleen cells from mice with sufficient titers of anti-HER3 immunoglobulin were frozen for later immortalization by fusion with mouse myeloma cell line P3X63 Ag8.653. One fusion was done and hybridoma supernatants screened by HER1/2/-ECD- ELISA showing no cross-reacivity, but binding to HER3-ECD and anti-HER3 selective hybridomas were selected. The relevant hybridomas were cloned by single cell FACS sorting. Single cell clones from different hybridomas were cultured in vitro to produce antibody in tissue culture medium for characterization. Antibodies were selectedby determining their ability to inhibit HER3 phosphorylation, AKT phosphorylation and tumor cell proliferation of MDA-MB-175 cells (see Examples below). From the obtained

antibodies, one was further humanized to give the following antibodies Mab 205.10.1, Mab 205.10.2 and Mab 205.10.3 with their respective VH and VL or CDRs.

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

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

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

Example 2

Binding assays

a) Antigene specific ELISA for binding to human HER3 ECD

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

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

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

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

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

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

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

Antibody	Binding Affinity KD [M]
Mab 205.10.1	$2.0x\ 10^{-9}$
Mab 205.10.2	$1.1x10^{-9}$
Mab 205.10.3	$2.0x10^{-9}$

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

Example 3

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

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

HER3 antibodies on receptor phosphorylation in MCF7 cells is shown below and in Fig 1A.

% Inhibition of HER3 phosphorylation in MCF7 cells

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

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

% Inhibition of HER3 phosphorylation in MCF7 cells

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

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

% Inhibition of HER3 phosphorylation in FaDu cells

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

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

% Inhibition of HER3 phosphorylation in Mel-Juso cells

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

b) AKT phosphorylation (ELISA)

Assays were performed in MCF7 cellsaccordign toth efollowing protocol:Seed MCF7 cells with 30000 cells/well into Poly-D-Lysine coated 96-well plate in RPMI1640 medium with 10% FCS and incubate for 24h. Remove medium by tapping on a clean paper towel, wash carefully with 200µl serum-free medium, incubate overnight with 100µl/well RPMI 1640 with 0.5% FCS. Remove medium as above; add antibodies in 100µl RPMI 1640 with 0.5% FCS and incubate 1.5h.

Add HRG-1b (final concentration 5ng/ml) for 10 min. Remove medium as above. To lyse the cells add 100µl ice cold cell lysis buffer on ice and resuspend by pipetting ca.5x. Centrifuge plate at 3000rpm for 10min at 4°C and transfer 80µl supernatant (or aliquots) into fresh polypropylene plate and shock-freeze in LN2. Store at -80°C until assay

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

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

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

% AKT phosporylation inhibition in Mel-Juso cells

Antibody	IC50 [µg/ml]
Mab 205.10.2	0.28
105.5 (α-HER^{ECD})	0.81
1B4C3	>50
2D1D12	>50
8B8D9	>50

c) Inhibition of tumor cell proliferation

The anti-tumor efficacy of HER3 antibodies Mab205.10.1, Mab205.10.2, and Mab205.10.3 in a cell proliferation assay, using MDA-MB-175 cells (VII Human Breast Carcinoma Cells, ATCC catalog no. HTB-25), was assessed. 20,000 cells per well were seeded into sterile 96 well tissue culture plates with DMEM/F12 cell culture medium, containing 10% FCS and incubated at 37°C±1°C with 5 % ±1% CO_2 for one day. The cells are slow growing cells with a doubling time of ca. 1.5

days. Anti-HER3 antibodies were added in dilution series and further incubated for 6 days. Cell viability was then assessed using the alamarBlue® readout. If the cell viability was reduced to more than 50 % of control, IC50 values were calculated using means of triplicates for each antibody concentration; otherwise, if the % inhibition of cell viability at the highest concentration was below 50%, no IC50 could be calculated and it is indicated that IC_{50} [µg/ml] is above (>) the highest concentration. Also the anti-HER3-antibodies U1-59 described in WO 2007/077028 and Ab#6 described in WO 2008/100624 were investigated.

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

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

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

Example 5

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

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

were washed three times with AIM-V medium, the cell number and viability was checked and the cell number adjusted to 0.3 Mio/ml.

Meanwhile, PBMC (Peripheral Blood Mononuclear Cells) as effector cells were prepared by density gradient centrifugation (Histopaque-1077, Sigma # H8889) according to the manufacturer's protocol (washing steps 1x at 400g and 2x at 350g 10 min each). The cell number and viability was checked and the cell number adjusted to 15 Mio/ml.

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

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

The killing of target cells was assessed by measuring LDH (Lactate Dehydrogenase) release from damaged cells using the Cytotoxicity Detection kit (LDH Detection Kit, Roche # 1 644 793) according to the manufacturer's instruction. Briefly, 100 µl supernatant from each well was mixed with 100 µl substrate from the kit in a transparent flat bottom 96 well plate. The Vmax values of the substrate's colour reaction was determined in an ELISA reader at 490 nm for at least 10 min. Percentage of specific antibody-mediated killing was calculated as follows: ((A – SR)/(MR – SR)x100, where A is the mean of Vmax at a specific antibody concentration, SR is the mean of Vmax of the spontaneous release and MR is the mean of Vmax of the maximal release.

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

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

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

The cells were incubated at 37°C, 5% CO2 for about 17 to 20 hours, then medium was replaced with 25 ml DMEM, 10% FCS. The conditioned culture medium was

harvested 7 days post-transfection by centrifugation for 15 min at 210 x g, the solution was sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % w/v was added, and kept at 4°C.

The secreted afucosylated antibodies were purified and the oligosaccharides attached to the Fc region of the antibodies were analysed e.g. by MALDI/TOF-MS (as described in e.g. WO 2008/077546). For this analysis oligosaccharides were enzymatically released from the antibodies by PNGaseF digestion, with the antibodies being either immobilized on a PVDF membrane or in solution. The resulting digest solution containing the released oligosaccharides either prepared directly for MALDI/TOF-MS analysis or was further digested with EndoH glycosidase prior to sample preparation for MALDI/TOF-MS analysis. The analyzed amount of fucose within the sugar chain at Asn297 was between 50-20%.

Example 6

In vivo antitumor efficacy

The in vivo antitumor efficacy of the antibodies Mab205.10.1, Mab205.10.2, Mab205.10.3 could be detected in cell and fragment based models of various tumor origin (e.g. lung cancer, SCCHN, breast- and pancreatic cancer) transplanted on SCID beige or nude mice. As examples data are shown for the SCCHN xenograft model FaDu (cell line based), breast cancer model MAXF449 (fragment-based) and NSCLC model 7177 (fragment-based).

Test agents

Afucosylated Mab205.10.2 (designated Mab 205 in Figures 2, 3, 4) was provided as stock solution from Roche, Penzberg, Germany. Antibody buffer included histidine. Antibody solution was diluted appropriately in buffer from stock prior injections.

Cell lines and culture conditions

FaDu human HNSCC cells were originally obtained from ATCC. The tumor cell line was routinely cultured in MEM Eagle medium supplemented with 10 % fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate and 0.1 mM NEAA at 37 °C in a water-saturated atmosphere at 5 % CO_2. Culture passage was performed with trypsin / EDTA 1x splitting every third day.

Tumor fragments

Tumor fragments were originally taken from patients and transplanted s.c. to nude donor mice. Subsequently tumor fragments are serial passaged in vivo. For a preclinical study small tumor fragments were generated from donor mice and placed s.c. on further nude mice (MAXF449, 7177).

Animals

Female SCID beige or nude mice were purchased from breeder (e.g. Charles River, Sulzfeld, Germany) and maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government. After arrival animals were maintained in the quarantine part of the animal facility for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis. Diet food (Provimi Kliba 3337) and water (acidified pH 2.5-3) were provided ad libitum.

Monitoring

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

Treatment of animals

Animal treatment started after animal randomisation after cell or fragment transplantation when median tumor size was about 100–150mm^3. Antibody was administered as single agent at 10 or 25mg/kg i.p. q7d once weekly for 3–6 weeks depending of the model. The corresponding vehicle was administered on the same days.

Antibody efficacy

A) FaDu HNSCC xenograft

FaDu HNSCC xenograft bearing mice were treated with antibody Mab205.10.2 from study day 14 to 35. As a result, treatment with the Mab205.10.2 antibody

showed significant anti-tumor efficacy with tumors stasis of s.c. FaDu xenografts. The Tumor Growth Inhibition (TGI) was calculated at 98%.

Treatment with Mab 205 (10mg/kg q7dx3, i.p.) resulted in tumor stasis of FaDu SCCHN transplanted xenografts (see Figure 2).

B) MAXF449 breast cancer xenograft

MAXF449 breast cancer xenograft bearing mice were treated with antibody Mab205.10.2 from study day 64 to 91. As a result, treatment with the Mab205.10.2 antibody showed significant anti-tumor efficacy with tumors stasis of MAXF449 xenografts. The Tumor Growth Inhibition (TGI) was over 100%.

Treatment with Mab 205 (10mg/kg q7d, i.p.) resulted in tumor stasis of MAXF449 breast cancer transplanted xenografts (see Figure 3).

C) 7177 NSCLC xenograft

7177 NSCLC xenograft bearing mice were treated with antibody Mab205.10.2 from study day 28 to 56. As a result, treatment with the Mab205.10.2 antibody showed significant anti-tumor efficacy with tumors stasis of 7177 NSCLC xenografts. The Tumor Growth Inhibition (TGI) was over 100%.

Treatment with Mab 205 (25mg/kg q7d, i.p.) resulted in tumor stasis of 7177 NSCLC transplanted xenografts (see Figure 4).

Patent Claims

1. An antibody which is binding to human HER3, characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9, or the light chain variable domain VL is SEQ ID NO:10, or the light chain variable domain VL is SEQ ID NO:11; or a humanized version thereof.

2. The antibody according to claim 1, characterized in that

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

3. An antibody according to claim 1, characterized in that the heavy chain variable domain VH is SEQ ID NO:8; and the light chain variable domain VL is SEQ ID NO:9, or the light chain variable domain VL is SEQ ID NO:11.

4. The antibody according to claims 1 to 3, characterized in that the antibody is monoclonal.

5. The antibody according to claims 1 to 4, characterized in that the antibody is of IgG1 subclass.

6. The antibody according to claims 1 to 5, characterized in that said antibody is glycosylated with a sugar chain at Asn297 whereby the amount of fucose within said sugar chain is 65 % or lower.

7. The antibody according to claim 6, whereby the amount of fucose within said sugar chain is 65 % or lower.

8. A pharmaceutical composition characterized by comprising an antibody according to claims 1 to 7.

9. An antibody according to claims 1 to 7 for the treatment of cancer.

10. Use of an antibody according to claims 1 to 7 for the manufacture of a medicament for the treatment of cancer.

11. A nucleic acid encoding a heavy and light chain of an antibody binding to human HER3, characterized in that said antibody comprises a variable domain according to claims 1 to 7.

12. An expression vector characterized in comprising a nucleic acid according to claim 11 for the expression of the antibody according to claims 1 to 7 in a prokaryotic or eukaryotic host cell.

13. A prokaryotic or eukaryotic host cell comprising a vector according to claim 12.

14. A method for the production of a recombinant antibody according to claims 1 to 7, characterized by expressing a nucleic acid according to claim 11 in a prokaryotic or eukaryotic host cell and recovering said antibody from said cell or the cell culture supernatant.