(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 September 2013 (06.09.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/127465 A1

(51) International Patent Classification:
G01N 33/574 (2006.01)

(21) International Application Number:
PCT/EP2012/053600

(22) International Filing Date:
2 March 2012 (02.03.2012)

(25) Filing Language: English

(26) Publication Language: English

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

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

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2013/127465 A1

(54) Title: PREDICITIVE BIOMARKER FOR CANCER TREATMENT WITH ADCC ENHANCED ANTIBODIES

(57) Abstract: The present invention is directed to methods for identifying which patients diagnosed with cancer will most benefit from treatment with an anti-cancer therapy comprising an ADCC-enhanced antibody.

PREDICITIVE BIOMARKER FOR CANCER TREATMENT WITH ADCC ENHANCED ANTIBODIES

Field of the invention

The present invention is directed to methods for identifying which patients diagnosed with cancer will most benefit from treatment with an anti-cancer therapy comprising an ADCC-enhanced antibody.

Background of the invention

ADCC-enhanced antibodies are an emerging species in the field of cancer therapy. It has been recognized that the so-called effector functions of an antibody, which are mediated by its Fc region, are an important mechanism of action in antibody-based cancer therapy. Of particular importance in this context is antibody-dependent cell-mediated cytotoxicity (ADCC), the destruction of antibody-coated target cells (e.g. tumor cells) by NK and other effector cells, which is triggered when antibody bound to the surface of a cell interacts with activating Fc receptors on the effector cell.

Enhancing the ADCC activity of therapeutic antibodies has therefore become of great interest and various methods for ADCC enhancement have been described. For example, Shields et al. (J Biol Chem 9(2), 6591-6604 (2001)) showed that amino acid substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues) improve ADCC. Alternatively, increased Fc receptor binding and effector function can be obtained by altering the glycosylation of an antibody. IgGl type antibodies, the most commonly used antibodies in cancer immunotherapy, have a conserved N-linked glycosylation site at Asn 297 in each CH2 domain of the Fc region. The two complex biantennary oligosaccharides attached to Asn 297 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 including ADCC (Lifely et al., Glycobiology 5, 813-822 (1995); Jefferis et al., Immunol Rev 163, 59-76 (1998); Wright and Morrison, Trends Biotechnol 15, 26-32 (1997)). Umaña et al. (Nat Biotechnol 17, 176-180 (1999) and U.S. Patent No. 6,602,684 (WO 99/54342), the contents of which are hereby incorporated by reference in their entirety) showed that overexpression of β(1,4)-N-

acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing the formation of bisected oligosaccharides, in Chinese hamster ovary (CHO) cells significantly increases the *in vitro* ADCC activity of antibodies produced in those cells. Overexpression of GnTIII in production cell lines leads to antibodies enriched in bisected oligosaccharides, which are generally also non-fucosylated and of the hybrid type. If in addition to GnTIII, mannosidase II (ManII) is overexpressed in production cell lines, antibodies enriched in bisected, non-fucosylated oligosaccharides of the complex type are obtained (Ferrara et al., Biotechn Bioeng 93, 851-861 (2006)). Both types of antibodies show strongly increased ADCC, as compared to antibodies with unmodified glycans, but only antibodies in which the majority of the N-glycans are of the complex type are able to induce significant complement-dependent cytotoxicity (Ferrara et al., Biotechn Bioeng 93, 851-861 (2006)). The elimination of fucose from the innermost N-acetylglucosamine residue of the oligosaccharide core appears to be the critical factor for the increase of ADCC activity (Shinkawa et al., J Biol Chem 278, 3466-3473 (2003)). Therefore, further methods for producing antibodies with reduced fucosylation were developed, including e.g. expression in α(1,6)-fucosyltransferase deficient host cells (Yamane-Ohnuki et al., Biotech Bioeng 87, 614-622 (2004); Niwa et al., J Immunol Methods 306, 151-160 (2006)).

Several ADCC-enhanced antibodies, including the glycoengineered anti-EGFR antibody <ge-huMabEGFR>, as well as the glycoengineered anti-CD20 antibody obinutuzumab, are currently in clinical development and have shown promising results. However, despite the great potential of ADCC-enhanced antibodies, in particular for cancer therapy, nothing is known so far on how to select patients which will most benefit from treatment with such antibodies. In view of the potential adverse effects associated with ineffective cancer therapies, it is generally acknowledged that there is a need for individualizing cancer treatment.

Therefore, it is an aim of the present invention to provide methods for determining which patients respond particularly well to ADCC-enhanced antibody therapy.

Summary of the invention

Investigations of the status of biomarkers related to anti-tumor immune responses revealed that the level of tumor infiltration by CD16+ cells prior to treatment with ADCC-enhanced antibodies correlated with an improved treatment outcome in several types of cancer.

The present invention is therefore related to a method of predicting the response of a cancer patient to treatment with an ADCC-enhanced antibody, comprising determining the level of

CD16+ cell infiltration in the tumor of the patient prior to treatment and comparing said level of CD16+ cell infiltration to a reference level, wherein a higher level of CD16+ cell infiltration compared to the reference level is indicative for a patient who will derive clinical benefit from the treatment.

In a further aspect the present invention provides an ADCC enhanced antibody for use in the treatment of cancer in a patient, wherein i) the level of CD16+ cell infiltration in the tumor of the patient is determined prior to treatment, ii) the level of CD16+ cell infiltration is compared to a reference level, and iii) the ADCC-enhanced antibody is administered to a patient having a higher level of CD16+ cell infiltration compared to the reference level.

The present invention also provides a method for the treatment of cancer in a patient, wherein i) the level of CD16+ cell infiltration in the tumor of the patient is determined prior to treatment, ii) the level of CD16+ cell infiltration is compared to a reference level, and iii) an ADCC-enhanced antibody is administered to a patient having a higher level of CD16+ cell infiltration compared to the reference level.

Further provided is a method of treating cancer in a patient comprising administering an effective amount of an ADCC-enhanced antibody to the patient, provided that the level of CD16+ cell infiltration in the tumor of the patient prior to treatment is higher than a reference level.

The present invention also relates to a pharmaceutical composition comprising an ADCC-enhanced antibody for the treatment of a cancer patient having an increased level of CD16+ cell infiltration in the tumor prior to treatment relative to a reference level.

In a further aspect the invention relates to a kit for detecting the level of CD16+ cell infiltration in a tumor, the kit comprising i) one or more compounds for detecting the level of CD16+ cell infiltration.

Detailed description of the invention

1. Definitions

The terms "administration" or "administering" as used herein mean the administration of a pharmaceutical composition, such as an ADCC-enhanced antibody, to a patient in need of such treatment or medical intervention by any suitable means known in the art. Nonlimiting routes of administration include by oral, intravenous, intraperitoneal, subcutaneous, intramuscular, topical,

intradermal, intranasal or intrabronchial administration (for example as effected by inhalation). Particularly preferred in context of this invention is parenteral administration, *e.g.*, intravenous administration.

The term "cancer" refers to the physiological condition in mammals that is typically characterized by unregulated cell proliferation. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma and leukemia. More particular examples of such cancers include squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer (including metastic pancreatic cancer), glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer (including locally advanced, recurrent or metastatic HER-2 negative breast cancer and locally recurrent or metastatic HER2 positive breast cancer), colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

The term "effective amount" refers to an amount of a drug alone or in combination with other drug or treatment regimen effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy in

vivo can, for example, be measured by assessing the duration of survival, duration of progression free survival (PFS), the response rates (RR), duration of response, and/or quality of life.

The term "overall survival (OS)" refers to the length of time during and after treatment the patient survives. As the skilled person will appreciate, a patient's overall survival is improved or enhanced, if the patient belongs to a subgroup of patients that has a statistically significant longer mean survival time as compared to another subgroup of patients.

The term "patient" refers to any single animal, more specifically a mammal (including such non-human animals as, for example, dogs, cats, horses, rabbits, zoo animals, cows, pigs, sheep, and non-human primates) for which treatment is desired. Even more specifically, the patient herein is a human.

The term "pharmaceutical composition" refers to a sterile preparation that is in such form as to permit the biological activity of the medicament to be effective, and which contains no additional components that are unacceptably toxic to a subject to which the formulation would be administered.

The term "progression-free survival (PFS)" refers to the length of time during and after treatment during which, according to the assessment of the treating physician or investigator, the patient's disease does not become worse, *i.e.,* does not progress. As the skilled person will appreciate, a patient's progression-free survival is improved or enhanced if the patient belongs to a subgroup of patients that has a longer length of time during which the disease does not progress as compared to the average or mean progression free survival time of a control group of similarly situated patients.

As used herein, “therapy” or “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.

A "value representative of the level of CD16+ cell infiltration in tumors of a population of patients deriving no clinical benefit from the treatment" refers to an estimate of a mean infiltration level of CD16+ cells in tumors of a population of patients who do not derive a clinical benefit from the treatment.

"Clinical benefit" is defined as having an objective response (e.g. according to RECIST criteria) or disease stabilization for at least 12 weeks.

The CD16 antigen is a cell surface antigen expressed on certain immune cells. It exists both as a transmembrane form (CD16a, Fcγ receptor IIIa), which is expressed e.g. on natural killer (NK) cells and activated macrophages, and as a glycosylphosphatidyl-inositol (GPI)-anchored form (CD16b, FcγRIIIb) expressed on neutrophils. "CD16" as used herein refers particularly to the CD16a antigen, also known as Fcγ receptor IIIa (see UniProt accession no. P08637 [version 136] and NCBI accession no. NP_000560 [version NP_000560.5] for the human protein). Accordingly, the term "CD16 positive cells" or "CD16+ cells" refers to cells expressing the CD16 antigen, particularly the CD16a antigen. CD16+ cells are detectable in a tissue sample for example by immunohistochemistry using an anti-CD16 antibody, such as the anti-CD16 antibody clone 2H7 (available from Biogenex).

The "level of CD16+ cell infiltration" refers to the number of CD16+ cells present in a given tissue, e.g. a tumor. In a particular embodiment, the level of CD16+ cell infiltration is reflected by the number of CD16+ cells per mm^2 of a tissue section (e.g. a section prepared from a tumor biopsy for the purpose of immunohistochemical analysis). In another embodiment, the level of CD16+ cell infiltration is reflected by the number of CD16+ cells per total number of cells in a tissue sample (e.g. a (part of) a tumor biopsy processed for flow cytometric analysis). In yet another embodiment, the level of CD16+ cell infiltration is reflected by the amount of CD16 protein or mRNA present in a tissue sample (e.g. a (part of) a tumor biopsy processed for ELISA analysis or a tissue sample processed for RT-PCT analysis).

By "prior to treatment" is meant before the first administration of ADCC-enhanced antibody to the patient.

"<ge-huMabEGFR>" refers to a glycoengineered, humanized IgG1-subclass anti-human EGFR antibody (CAS Registry Number 959963-46-3) based on the rat ICR62 antibody (Modjtahedi et al. (1996), Br J Cancer 73, 228-235). The antibody is produced in host cells overexpressing polypeptides having β(1,4)-N-acetylglucosaminyltransferase III (GnTIII) and mannosidase II

(ManII) activity (see Umaña et al. (1999) Nat Biotechnol 17, 176-180 and U.S. Patent No. 6,602,684 (WO 99/54342), Ferrara et al. (2006) Biotechn Bioeng 93, 851-861) and has at least about 50% non-fucosylated oligosaccharides in its Fc region.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity and comprise an Fc region or a region equivalent to the Fc region of an immunoglobulin.

The terms “full-length antibody”, “intact antibody”, and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain, also called a light chain constant region. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab’-SH, $F(ab')_2$, diabodies, linear antibodies, single-chain antibody molecules (e.g. scFv), single-domain antibodies, and multispecific antibodies formed from antibody fragments. For a review of certain antibody fragments, see Hudson et al., Nat Med 9, 129-134 (2003). For a review of scFv fragments, see e.g. Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No.

5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity.

The term "hypervariable region" or "HVR", as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the complementarity determining regions (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. Hypervariable regions (HVRs) are also referred to as "complementarity determining regions" (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, Sequences of Proteins of Immunological Interest (1983) and by Chothia et al., J Mol Biol 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The

appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE 1. CDR Definitions[1]

CDR	Kabat	Chothia	AbM[2]
V_H CDR1	31-35	26-32	26-35
V_H CDR2	50-65	52-58	50-58
V_H CDR3	95-102	95-102	95-102
V_L CDR1	24-34	26-32	24-34
V_L CDR2	50-56	50-52	50-56
V_L CDR3	89-97	91-96	89-97

[1] Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat et al. (see below).

[2] "AbM" with a lowercase "b" as used in Table 1 refers to the CDRs as defined by Oxford Molecular's "AbM" antibody modeling software.

Kabat et al. also defined a numbering system for variable region sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable region sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody variable region are according to the Kabat numbering system.

The polypeptide sequences of the sequence listing (i.e., SEQ ID NOs 1-9.) are not numbered according to the Kabat numbering system. However, it is well within the ordinary skill of one in the art to convert the numbering of the sequences of the Sequence Listing to Kabat numbering.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

A "region equivalent to the Fc region of an immunoglobulin" is intended to include naturally occurring allelic variants of the Fc region of an immunoglobulin as well as variants having alterations which produce substitutions, additions, or deletions but which do not decrease substantially the ability of the immunoglobulin to mediate effector functions (such as antibody-dependent cell-mediated cytotoxicity). For example, one or more amino acids can be deleted from the N-terminus or C-terminus of the Fc region of an immunoglobulin without substantial loss of biological function. Such variants can be selected according to general rules known in the art so as to have minimal effect on activity (see, e.g., Bowie et al., Science 247, 1306-10 (1990)).

The terms "anti-[antigen] antibody" and "an antibody that binds to [antigen]" refer to an antibody that is capable of binding the respective antigen with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting the antigen. In one embodiment, the extent of binding of an anti-[antigen] antibody to an unrelated protein is less

than about 10% of the binding of the antibody to the antigen as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to [antigen] has a dissociation constant (K_D) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 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).

As used herein, the terms "engineer, engineered, engineering" are considered to include any manipulation of the peptide backbone or the post-translational modifications of a naturally occurring or recombinant polypeptide or fragment thereof. Engineering includes modifications of the amino acid sequence, of the glycosylation pattern, or of the side chain group of individual amino acids, as well as combinations of these approaches. "Engineering", particularly with the prefix "glyco-", as well as the term "glycosylation engineering" includes metabolic engineering of the glycosylation machinery of a cell, including genetic manipulations of the oligosaccharide synthesis pathways to achieve altered glycosylation of glycoproteins expressed in cells. Furthermore, glycosylation engineering includes the effects of mutations and cell environment on glycosylation. In one embodiment, the glycosylation engineering is an alteration in glycosyltransferase activity. In a particular embodiment, the engineering results in altered glucosaminyltransferase activity and/or fucosyltransferase activity. Glycosylation engineering can be used to obtain a "host cell having increased GnTIII activity" (e.g. a host cell that has been manipulated to express increased levels of one or more polypeptides having β(1,4)-N-acetylglucosaminyltransferase III (GnTIII) activity), a "host cell having increased ManII activity" (e.g. a host cell that has been manipulated to express increased levels of one or more polypeptides having α-mannosidase II (ManII) activity), or a "host cell having decreased α(1,6) fucosyltransferase activity" (e.g. a host cell that has been manipulated to express decreased levels of α(1,6) fucosyltransferase). A host cell is any type of cellular system that can be used to generate ADCC-enhanced antibodies. Host cells include cultured cells, *e.g.* mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

The host cells which contain the coding sequence of an antibody useful in the context the invention and/or the coding sequence of polypeptides having glycosyltransferase activity, and which express the biologically active gene products may be identified e.g. by DNA-DNA or DNA-RNA hybridization; the presence or absence of "marker" gene functions; assessing the level of transcription as measured by the expression of the respective mRNA transcripts in the

host cell; or detection of the gene product as measured by immunoassay or by its biological activity - methods which are well known in the art. GnTIII or Man II activity can be detected e.g. by employing a lectin which binds to biosynthetis products of GnTIII or ManII, respectively. An example for such a lectin is the E_4-PHA lectin which binds preferentially to oligosaccharides containing bisecting GlcNAc. Biosynthesis products (i.e. specific oligosaccharide structures) of polypeptides having GnTIII or ManII activity can also be detected by mass spectrometric analysis of oligosaccharides released from glycoproteins produced by cells expressing said polypeptides. Alternatively, a functional assay which measures the increased effector function, e.g. increased ADCC, mediated by antibodies produced by the cells engineered with the polypeptide having GnTIII or ManII activity may be used.

As used herein, the term "polypeptide having GnTIII activity" refers to polypeptides that are able to catalyze the addition of a N-acetylglucosamine (GlcNAc) residue in β-1,4 linkage to the β-linked mannoside of the trimannosyl core of N-linked oligosaccharides. This includes fusion polypeptides exhibiting enzymatic activity similar to, but not necessarily identical to, an activity of β(1,4)-N-acetylglucosaminyltransferase III, also known as β-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyl-transferase (EC 2.4.1.144), according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of GnTIII, but rather substantially similar to the dose-dependency in a given activity as compared to the GnTIII (i.e. the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about ten-fold less activity, and most preferably, not more than about three-fold less activity relative to the GnTIII). In certain embodiments the polypeptide having GnTIII activity is a fusion polypeptide comprising the catalytic domain of GnTIII and the Golgi localization domain of a heterologous Golgi resident polypeptide. Particularly, the Golgi localization domain is the localization domain of mannosidase II or GnTI, most particularly the localization domain of mannosidase II. Alternatively, the Golgi localization domain is selected from the group consisting of: the localization domain of mannosidase I, the localization domain of GnTII, and the localization domain of α1,6 core fucosyltransferase. Methods for generating such fusion polypeptides and using them to produce antibodies with increased effector functions are disclosed in WO2004/065540, U.S. Provisional Pat. Appl. No. 60/495,142 and U.S. Pat. Appl. Publ. No. 2004/0241817, the entire contents of which are expressly incorporated herein by reference.

As used herein, the term “Golgi localization domain” refers to the amino acid sequence of a Golgi resident polypeptide which is responsible for anchoring the polypeptide to a location within the Golgi complex. Generally, localization domains comprise amino terminal "tails" of an enzyme.

As used herein, the term "polypeptide having ManII activity" refers to polypeptides that are able to catalyze the hydrolysis of the terminal 1,3- and 1,6-linked α-D-mannose residues in the branched $GlcNAcMan_5GlcNAc_2$ mannose intermediate of N-linked oligosaccharides. This includes polypeptides exhibiting enzymatic activity similar to, but not necessarily identical to, an activity of Golgi α-mannosidase II, also known as mannosyl oligosaccharide 1,3-1,6-α-mannosidase II (EC 3.2.1.114), according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB).

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism leading to the lysis of antibody-coated target cells by immune effector cells. The target cells are cells to which antibodies or fragments thereof comprising an Fc region specifically bind, generally via the protein part that is N-terminal to the Fc region. As used herein, the term “increased ADCC” is defined as either an increase in the number of target cells that are lysed in a given time, at a given concentration of antibody in the medium surrounding the target cells, by the mechanism of ADCC defined above, and/or a reduction in the concentration of antibody, in the medium surrounding the target cells, required to achieve the lysis of a given number of target cells in a given time, by the mechanism of ADCC. The increase in ADCC is relative to the ADCC mediated by the same antibody produced by the same type of host cells, using the same standard production, purification, formulation and storage methods (which are known to those skilled in the art), but that has not been engineered. For example the increase in ADCC mediated by an antibody produced by host cells engineered to have an altered pattern of glycosylation (e.g. to express the glycosyltransferase, GnTIII, or other glycosyltransferases) by the methods described herein, is relative to the ADCC mediated by the same antibody produced by the same type of non-engineered host cells.

By “antibody having increased antibody dependent cell-mediated cytotoxicity (ADCC)” is meant an antibody having increased ADCC as determined by any suitable method known to those of ordinary skill in the art. One accepted *in vitro* ADCC assay is as follows:

1) the assay uses target cells that are known to express the target antigen recognized by the antigen-binding region of the antibody;

2) the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;

3) the assay is carried out according to following protocol:

i) the PBMCs are isolated using standard density centrifugation procedures and are suspended at 5 x 10^6 cells/ml in RPMI cell culture medium;

ii) the target cells are grown by standard tissue culture methods, harvested from the exponential growth phase with a viability higher than 90%, washed in RPMI cell culture medium, labeled with 100 micro-Curies of ^{51}Cr, washed twice with cell culture medium, and resuspended in cell culture medium at a density of 10^5 cells/ml;

iii) 100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;

iv) the antibody is serially-diluted from 4000 ng/ml to 0.04 ng/ml in cell culture medium and 50 microliters of the resulting antibody solutions are added to the target cells in the 96-well microtiter plate, testing in triplicate various antibody concentrations covering the whole concentration range above;

v) for the maximum release (MR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of a 2% (V/V) aqueous solution of non-ionic detergent (Nonidet, Sigma, St. Louis), instead of the antibody solution (point iv above);

vi) for the spontaneous release (SR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of RPMI cell culture medium instead of the antibody solution (point iv above);

vii) the 96-well microtiter plate is then centrifuged at 50 x g for 1 minute and incubated for 1 hour at 4°C;

viii) 50 microliters of the PBMC suspension (point i above) are added to each well to yield an effector:target cell ratio of 25:1 and the plates are placed in an incubator under 5% CO_2 atmosphere at 37°C for 4 hours;

ix) the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;

x) the percentage of specific lysis is calculated for each antibody concentration according to the formula (ER-MR)/(MR-SR) x 100, where ER is the average radioactivity quantified (see point ix above) for that antibody concentration, MR is the average radioactivity quantified (see point ix above) for the MR controls (see point v above), and SR is the average radioactivity quantified (see point ix above) for the SR controls (see point vi above);

4) "increased ADCC" is defined as either an increase in the maximum percentage of specific lysis observed within the antibody concentration range tested above, and/or a reduction in the concentration of antibody required to achieve one half of the maximum percentage of specific lysis observed within the antibody concentration range tested above. The increase in ADCC is relative to the ADCC, measured with the above assay, mediated by the same antibody, produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, which are known to those skilled in the art, but that has not been engineered.

Other examples of *in vitro* assays to assess ADCC activity of an antibody are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the antibody may be assessed in vivo, e.g. in an animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

2. Detailed Embodiments

In a first aspect, the present invention provides a method of predicting the response of a cancer patient to treatment with an ADCC-enhanced antibody, comprising determining the level of CD16+ cell infiltration in the tumor of the patient prior to treatment and comparing said level of CD16+ cell infiltration to a reference level, wherein a higher level of CD16+ cell infiltration compared to the reference level is indicative for a patient who will derive clinical benefit from the treatment.

In certain embodiments, the method is an in vitro method. In one such embodiment, the level of CD16+ cell infiltration is determined in a tumor sample taken from the patient prior to treatment. In one embodiment, the reference level is a value representative of the level of CD16+ cell infiltration in tumors of a population of patients deriving no clinical benefit from the treatment. In one embodiment, the reference level is determined in vitro in tumor samples taken prior to treatment from patients deriving no clinical benefit from the treatment. In one embodiment, the level of CD16+ cell infiltration which is indicative for a patient who will derive clinical benefit

from the treatment is at least 1.2-fold, at least 1.5-fold, at least 2-fold or at least 3-fold higher than the reference level.

In a further aspect the present invention provides an ADCC-enhanced antibody for use in the treatment of cancer in a patient, wherein i) the level of CD16+ cell infiltration in the tumor of the patient is determined prior to treatment, ii) the level of CD16+ cell infiltration is compared to a reference level, and iii) the ADCC-enhanced antibody is administered to a patient having a higher level of CD16+ cell infiltration compared to the reference level.
In one embodiment, the level of CD16+ cell infiltration in the tumor is determined in vitro in a tumor sample taken from the patient prior to treatment. In one embodiment, the reference level is a value representative of the level of CD16+ cell infiltration in tumors of a population of patients deriving no clinical benefit from the treatment. In one embodiment, the reference level is determined in vitro in tumor samples taken prior to treatment from patients deriving no clinical benefit from the treatment. In one embodiment, the ADCC-enhanced antibody is administered to a patient having an at least 1.2-fold, at least 1.5-fold, at least 2-fold or at least 3-fold higher level of CD16+ cell infiltration compared to the reference level.

The present invention also provides a method for the treatment of cancer in a patient, wherein i) the level of CD16+ cell infiltration in the tumor of the patient is determined prior to treatment, ii) the level of CD16+ cell infiltration is compared to a reference level, and iii) an ADCC-enhanced antibody is administered to a patient having a higher level of CD16+ cell infiltration compared to the reference level.
In one embodiment, the level of CD16+ cell infiltration in the tumor is determined in vitro in a tumor sample taken from the patient prior to treatment. In one embodiment, the reference level is a value representative of the level of CD16+ cell infiltration in tumors of a population of patients deriving no clinical benefit from the treatment. In one embodiment, the reference level is determined in vitro in tumor samples taken prior to treatment from patients deriving no clinical benefit from the treatment. In one embodiment, the ADCC-enhanced antibody is administered to a patient having an at least 1.2-fold, at least 1.5-fold, at least 2-fold or at least 3-fold higher level of CD16+ cell infiltration compared to the reference level.

Further provided is a method of treating cancer in a patient comprising administering an effective amount of an ADCC-enhanced antibody to the patient, provided that the level of CD16+ cell infiltration in the tumor of the patient prior to treatment is higher than a reference level.

In one embodiment, the level of CD16+ cell infiltration in the tumor is the level determined in vitro in a tumor sample taken from the patient prior to treatment. In one embodiment, the reference level is a value representative of the level of CD16+ cell infiltration in tumors of a population of patients deriving no clinical benefit from the treatment. In one embodiment, the reference level is determined in vitro in tumor samples taken prior to treatment from patients deriving no clinical benefit from the treatment. In one embodiment, the level of CD16+ cell infiltration is at least 1.2-fold, at least 1.5-fold, at least 2-fold or at least 3-fold higher compared to the reference level.

In one aspect, the present invention also provides a pharmaceutical composition comprising an ADCC-enhanced antibody for the treatment of a cancer patient having an increased level of CD16+ cell infiltration in the tumor prior to treatment relative to a reference level.

In one embodiment, the level of CD16+ cell infiltration in the tumor is determined in vitro in a tumor sample taken from the patient prior to treatment.

In one embodiment, the reference level is a value representative of the level of CD16+ cell infiltration in tumors of a population of patients deriving no clinical benefit from the treatment. In one embodiment, the reference level is determined in vitro in tumor samples taken prior to treatment from patients deriving no clinical benefit from the treatment. In one embodiment, the patient has an at least 1.2-fold, at least 1.5-fold, at least 2-fold or at least 3-fold increased level of CD16+ cell infiltration relative to the reference level.

In a further aspect the invention relates to a kit for detecting the level of CD16+ cell infiltration in a tumor, the kit comprising i) one or more compounds for detecting the level of CD16+ cell infiltration. In certain embodiments, the kit further comprises ii) instructions for using said kit to predict responsiveness of a cancer patient to treatment with an ADCC-enhanced antibody, wherein a higher level of CD16+ cell infiltration in the tumor of the patient prior to treatment, compared to a reference value, is indicative for a patient who will derive clinical benefit from the treatment.

In certain embodiments, the kit is for in vitro use. In one such embodiment, the level of CD16+ cell infiltration is detected in a tumor sample taken from a cancer patient prior to treatment with an ADCC-enhanced antibody. In one embodiment, the reference level is a value representative of the level of CD16+ cell infiltration in tumors of a population of patients deriving no clinical benefit from the treatment. In one embodiment, the reference level is determined in vitro in tumor samples taken prior to treatment from patients deriving no clinical benefit from the

treatment. In one embodiment, the level of CD16+ cell infiltration which is indicative for a patient who will derive clinical benefit from the treatment is at least 1.2-fold, at least 1.5-fold, at least 2-fold or at least 3-fold higher than the reference level.

3. ADCC-enhanced antibodies

An ADCC-enhanced antibody as defined herein for the various aspects of the present invention is an antibody engineered to have increased ADCC-activity as compared to a corresponding non-engineered antibody. In particular embodiments, the ADCC-enhanced antibody is a glycoengineered antibody comprising an increased proportion of non-fucosylated oligosaccharides in its Fc region, compared to a non-glycoengineered antibody. In one such embodiment, the antibody is produced in a host cell engineered to have increased β(1,4)-N-acetylglucosaminyltransferase III (GnTIII) activity, compared to a non-engineered host cell. In a more specific embodiment the host cell additionally is engineered to have increased α-mannosidase II (ManII) activity, compared to a non-engineered host cell. A host cell may be engineered to have increased β(1,4)-N-acetylglucosaminyltransferase III (GnTIII) activity by overexpression of one or more polypeptides having β(1,4)-N-acetylglucosaminyltransferase III (GnTIII) activity. Likewise, a host cell may be engineered to have increased α-mannosidase II (ManII) activity by overexpression of one or more polypeptides having α-mannosidase II (ManII) activity. This glycoengineering methodology has been described in greater detail in Umana et al., Nat Biotechnol 17, 176-180 (1999); Ferrara et al., Biotechn Bioeng 93, 851-861 (2006); WO 99/54342 (U.S. Pat. No. 6,602,684; EP 1071700); WO 2004/065540 (U.S. Pat. Appl. Publ. No. 2004/0241817; EP 1587921), WO 03/011878 (U.S. Pat. Appl. Publ. No. 2003/0175884), the entire content of each of which is incorporated herein by reference in its entirety.

In an alternative embodiment the ADCC-enhanced antibody is a glycoengineered antibody comprising an increased proportion of non-fucosylated oligosaccharides in its Fc region, compared to a non-glycoengineered antibody, wherein the antibody is produced in a host cell having decreased α(1,6)-fucosyltransferase activity. A host cell having decreased α(1,6)-fucosyltransferase activity may be a cell in which the α(1,6)-fucosyltransferase gene has been disrupted or otherwise deactivated, e.g. knocked out (see Yamane-Ohnuki et al., Biotech Bioeng 87, 614 (2004); Kanda et al., *Biotechnol Bioeng*, 94(4), 680-688 (2006); Niwa et al., J Immunol Methods 306, 151-160 (2006)).

In one embodiment, the ADCC-enhanced antibody is an antibody having at least about 50% non-fucosylated oligosaccharides in its Fc region. In one embodiment, the ADCC enhanced antibody is an antibody having at least about 75% non-fucosylated oligosaccharides in its Fc region. In another embodiment, the ADCC-enhanced antibody is an antibody having at least about 50% bisected oligosaccharides in its Fc region. In one embodiment, the ADCC enhanced antibody is an antibody having at least about 50% bisected, non-fucosylated oligosaccharides in its Fc region. The oligosaccharide structures in the antibody Fc region can be analysed by methods well known in the art, e.g. by MALDI TOF mass spectrometry as described in Umana et al., Nat Biotechnol 17, 176-180 (1999) or Ferrara et al., Biotechn Bioeng 93, 851-861 (2006). The percentage of non-fucosylated oligosaccharides is the amount of oligosaccharides lacking fucose residues, relative to all oligosaccharides attached to Asn 297 (e. g. complex, hybrid and high mannose structures) and identified in an N-glycosidase F treated sample by MALDI TOF MS. Asn 297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. The percentage of bisected, or bisected non-fucosylated, oligosaccharides is determined analogously.

In one embodiment the ADCC-enhanced antibody is a full-length antibody of the IgG-class. In a particular embodiment, the ADCC-enhanced antibody is an IgG1 antibody. In one embodiment, the ADCC-enhanced antibody comprises a human Fc region, more particularly a human IgG Fc region, most particularly a human IgG1 Fc region. The ADCC-enhanced antibodies may comprise a human Ig gamma-1 heavy chain constant region, as set forth in SEQ ID NO: 1 (i.e. the antibodies are of human IgG1 subclass).

In certain embodiments the ADCC-enhanced antibody is directed to an antigen presented on a tumor cell. Particular target antigens of the ADCC-enhanced antibodies in the context of the present invention include antigens expressed on the surface of tumor cells, including, but not limited to, cell surface receptors such as epidermal growth factor receptor (EGFR), insulin-like growth factor receptors (IGFR) and platelet-derived growth factor receptors (PDGFR), prostate specific membrane antigen (PSMA), carcinoembryonic antigen (CEA), dipeptidyl peptidase IV (CD26, DPPIV), fibroblast activation protein (FAP), HER2/neu, HER-3, E-cadherin, CD20, melanoma-associated chondroitin sulfate proteoglycan (MCSP), c-Met, CUB domain-containing protein-1 (CDCP1), and squamous cell carcinoma antigen (SCCA).

In one embodiment, the ADCC-enhanced antibody is directed to an antigen selected from the group of CD20, EGFR, HER2, HER3, IGF-1R, CEA, c-Met, CDCP1, FAP and MCSP. In one embodiment, the ADCC-enhanced antibody is a multispecific antibody directed to two or more antigens selected from the group of CD20, EGFR, HER2, HER3, IGF-1R, CEA, c-Met, CDCP1, FAP and MCSP.

In a particular embodiment, the ADCC-enhanced antibody is an anti-EGFR antibody, more particularly an anti-human EGFR antibody. Suitable ADCC-enhanced anti-EGFR antibodies are described in WO 2006/082515 and WO 2008/017963, each of which is incorporated herein by reference in its entirety.

In a specific embodiment, the ADCC-enhanced antibody is a humanized, IgG1-subclass anti-EGFR antibody comprising a) in the heavy chain variable domain a CDR1 of SEQ ID NO: 2, a CDR2 of SEQ ID NO: 3, and a CDR3 of SEQ ID NO: 4, and b) in the light chain variable domain a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6, and a CDR3 of SEQ ID NO: 7.

In an even more specific embodiment, the ADCC-enhanced antibody is a humanized, IgG1-subclass anti-EGFR antibody comprising the heavy chain variable domain of SEQ ID NO: 8 and the light chain variable domain of SEQ ID NO: 9.

In another specific embodiment, the ADCC-enhanced antibody is a humanized, IgG1-subclass anti-EGFR antibody comprising a) in the heavy chain variable domain a CDR1 of SEQ ID NO: 2, a CDR2 of SEQ ID NO: 3, and a CDR3 of SEQ ID NO: 4, and b) in the light chain variable domain a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6, and a CDR3 of SEQ ID NO: 7, wherein the antibody is glycoengineered to have an increased proportion of non-fucosylated oligosaccharides it its Fc region compared to a corresponding non-glycoengineered antibody.

In a more specific embodiment, the ADCC-enhanced antibody is a humanized, IgG1-subclass anti-EGFR antibody comprising the heavy chain variable domain of SEQ ID NO: 8 and the light chain variable domain of SEQ ID NO: 9, wherein the antibody is glycoengineered to have an increased proportion of non-fucosylated oligosaccharides it its Fc region compared to a corresponding non-glycoengineered antibody.

In yet another specific embodiment, the ADCC-enhanced antibody is a humanized, IgG1-subclass anti-EGFR antibody comprising a) in the heavy chain variable domain a CDR1 of SEQ ID NO: 2, a CDR2 of SEQ ID NO: 3, and a CDR3 of SEQ ID NO: 4, and b) in the light chain variable domain a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6, and a CDR3 of SEQ ID NO: 7, and c) in the Fc region at least 50% non-fucosylated oligosaccharides.

In a more specific embodiment, the ADCC-enhanced antibody is a humanized, IgG1-subclass anti-EGFR antibody comprising the heavy chain variable domain of SEQ ID NO: 8, the light chain variable domain of SEQ ID NO: 9, the heavy chain constant region of SEQ ID NO: 1, and at least 50% non-fucosylated oligosaccharides in the Fc region.

In one embodiment the ADCC-enhanced antibody is <ge-huMabEGFR>.

4. Cancer types

The present invention is useful in different types of cancer. In particular embodiments according to the invention, the cancer is a solid tumor. In one such embodiment, the cancer is selected from the group consisting of colorectal cancer, lung cancer, head and neck cancer, breast cancer, pancreatic cancer, renal cancer, ovarian cancer, gastric cancer and skin cancer. In one embodiment, the cancer is non-small cell lung carcinoma (NSCLC). In a more specific embodiment, the cancer is non-squamous NSCLC. In another embodiment, the cancer is colorectal carcinoma (CRC). In yet another embodiment, the cancer is head and neck squamous cell carcinoma (HNSCC). In one embodiment the cancer is selected from the group consisting of NSCLC, CRC and HNSCC.

5. Combinations of markers

The biomarker of the present invention, i.e. the level of CD16+ cell infiltration prior to treatment, can be combined with other biomarkers to biomarker sets. Biomarker sets can be built from any combination of predictive biomarkers to make predictions about the effect of ADCC-enhanced antibody treatment in cancer patients. For example, the level of CD16+ cell infiltration prior to treatment might be combined with the expression level of the target antigen of the ADCC-enhanced antibody to a biomarker set.

6. Determination of infiltration levels of CD16+ cells

The level of CD16+ cell infiltration may be assessed by any method known in the art suitable for visualizing cells in patient tissues, such as immunohistochemical or immunofluorescent methods using anti-CD16 antibodies. In a particular embodiment, the level of CD16+ cell infiltration is determined by immunohistochemistry. A suitable anti-CD16 antibody that can be used for detection of CD16+ cell by immunohistochemistry is the anti-CD16 antibody clone 2H7.

In an alternative embodiment, the level of CD16+ cell infiltration is determined by flow cytometry. Flow cytometric methods (FACS) are well known in the art for the quantification of

cells in tissue samples. In particular, they allow determining the number of cells expressing a specific antigen (e.g. CD16+ cells) among a defined total number of cells in a tissue sample (e.g. a (part of) a tumor biopsy).

The level of CD16+ cell infiltration may also be determined indirectly, by quantification of CD16 protein or mRNA levels in patient tissues. Suitable methods known in the art for the determination of specific protein levels include immunoassay methods such as enzyme-linked immunosorbent assay (ELISA), methods for determination of mRNA levels include for example quantitative RT-PCR or microarray technologies.

All the above mentioned methods and technologies are well known in the art and can be deduced from standard textbooks such as Lottspeich (Bioanalytik, Spektrum Akademisher Verlag, 1998) or Sambrook and Russell (Molecular Cloning: A Laboratory Manual, CSH Press, Cold Spring Harbor, NY, U.S.A., 2001).

7. Methods of treatment

In the context of the present invention, <ge-huMabEGFR> is to be administered alone or in addition to or as a co-therapy or co-treatment with one or more chemotherapeutic agents administered as part of standard chemotherapy regimen as known in the art. Examples of agents included in such standard chemotherapy regimens include 5-fluorouracil, leucovorin, irinotecan, gemcitabine, erlotinib, capecitabine, taxanes, such as docetaxel and paclitaxel, interferon alpha, vinorelbine, and platinum-based chemotherapeutic agents, such as, carboplatin, cisplatin and oxaliplatin.

Common modes of administration include parenteral administration as a bolus dose or as an infusion over a set period of time, e.g., administration of the total daily dose over 10 min., 20 min., 30 min., 40 min., 50 min., 60 min., 75 min., 90 min., 105 min., 120 min., 3 hr., 4 hr., 5 hr. or 6 hr. For example, 2.5 mg/kg of body weight to 25 mg/kg of body weight <ge-huMabEGFR> can be administered every week, every 2 weeks or every 3 weeks, depending on the type of cancer being treated. Examples of dosages include 2.5 mg/kg of body weight, 5 mg/kg of body weight, 7.5 mg/kg of body weight, 10 mg/kg of body weight, 15 mg/kg of body weight, 20 mg/kg of body weight and 25 mg/kg of body weight given every week, every 2 weeks or every 3 weeks. Further examples of dosages are 700 mg every 2 weeks, 1000 mg every 2 weeks, 1400 mg every two weeks, 700 mg every 3 weeks, 1000 mg every 3 weeks, and 1400 mg every 3 weeks.

The skilled person will recognize that further modes of administration and dosages of <ge-huMabEGFR> are encompassed by the invention as determined by the specific patient and chemotherapy regimen, and that the specific mode of administration and therapeutic dosage are best determined by the treating physician according to methods known in the art.

8. Kit

The present invention also relates to a diagnostic composition or kit comprising one or more compounds for detecting the level of CD16+ cell infiltration. As detailed herein, anti-CD16 antibodies may be of use for detecting CD16 protein and may thus be comprised in a kit according to the invention. Such antibodies may be labelled (e.g. with a fluorescent label, a radiolabel, an enzymatic label or a biotin/avidin complex) to enable their direct detection, or may be used in combination with labelled secondary antibodies (i.e. antibodies that specifically bind to specific other antibodies such as antibodies from a particular host species). Appropriate secondary antibodies may thus also be comprised in the kit. Further components may be reagents needed for carrying out the detection, e.g. buffers, fixatives, blocking reagents, diluents, chromogens, enzymes etc. for immunohistochemistry, immunofluorescence, ELISA, Western Blotting or whatever the detection method of choice may be. Alternatively, if CD16 mRNA is to be detected, the kit may comprise oligonucleotides such as primers and fluorescent probes for real-time PCR, enzymes for preparation of cDNA such as reverse transcriptase, and the like.

In a further aspect of the invention, the kit of the invention may advantageously be used for carrying out a method of the invention and could be, inter alia, employed in a variety of applications, e.g., in the diagnostic field or as a research tool. The parts of the kit of the invention can be packaged individually in vials or in combination in containers or multicontainer units. Manufacture of the kit follows preferably standard procedures which are known to the person skilled in the art. The kit or diagnostic compositions may be used for detection of the level of CD16+ cell infiltration in tumors in accordance with the herein-described methods of the invention, employing, for example, immunohistochemical techniques described herein.

Brief description of the drawings

FIGURE 1. Figure 1 shows the correlation between the percent change of the sum of longest diameter (SLD) from baseline to the first post-baseline tumour assessment at 8 weeks and baseline staining in new tumour biopsies obtained at baseline for CD16+ cells.

FIGURE 2. Figure 2 shows the correlation between the percent change of the sum of longest diameter (SLD) from baseline to the second post-baseline tumour assessment at 12 weeks and baseline staining in tumour biopsies obtained at baseline for CD16+ cells.

FIGURE 3. Figure 3 shows the correlation between the SUV_{max} reduction from baseline to the post-baseline tumour assessment at 2 weeks and baseline staining in new tumour biopsies obtained at baseline for CD16+ cells.

Examples

The present invention is further illustrated by the following non-limiting illustrative examples.

Example 1: Colorectal carcinoma (CRC)

Study design

This was the second part of an open-label, multicentre, non-randomised, dose-escalating, phase I study of <ge-huMabEGFR> in patients with solid tumors. In this part of the study 25 patients with *KRAS*-mutant colorectal carcinoma (CRC) were treated, based on the safety profile and efficacy demonstrated in the first part of the study (Paz Ares et al. (2011), J Clin Oncol 29, 3783-90) with a flat dose of 1400 mg <ge-huMabEGFR> administered intravenously (i.v.) on days 1 and 8 followed by dosing every 2 weeks (q2W).

Patient selection

Eligible patients were aged ≥18 years with an Eastern Cooperative Oncology Group performance status of ≤1 and adequate haematology, blood chemistry, renal and liver function. Patients had histologically/cytologically confirmed metastatic EGFR-positive and *KRAS*-mutant CRC (codons 12/13/61) with radiologically measurable progressive disease (PD). Patients with more than two previous cytotoxic regimens for metastatic disease were excluded. All patients gave written informed consent and approval from local Ethics Committees was obtained. The study was conducted in accordance with Good Clinical Practice guidelines.

Administration of the drug

The first dose of <ge-huMabEGFR> was administered at an initial infusion rate of 10 mg/hour. After one hour, infusion rates were escalated every 30 minutes up to 800 mg/hour. Subsequent infusions began at 20 mg/hour if the first infusion was well tolerated. Premedication with paracetamol, anti-histamine and corticosteroids was given for the first two infusions, to minimise the risk of infusion-related reactions (IRRs).

Tumor biopsies

Optional tumour biopsies for immunohistochemistry (HistoGeneX) were taken pre-treatment at baseline. Eleven patients enrolled in to the optional biomarker program, providing a tumor biopsy prior (generally not more than two weeks) to the first <ge-huMabEGFR> infusion. Biopsies were formalin-fixed and paraffin-embedded, and analysed for immune effector cell infiltrates by immunohistochemistry (IHC). Tumour-infiltrating immune effector cells were graded by counting the number of positive staining cells/mm^2.

Immunohistochemical analysis

IHC was performed on a Ventana Benchmark XT system. Deparaffinized slides were incubated for 1 hour with the anti-CD16 antibody clone 2H7 (Biogenex) diluted 1:10 in antibody diluent (Ventana #251-018). The ultra view™ Universal DAB detection kit (Ventana) was used for detection, followed by 4 minutes incubation with hematoxylin II (Ventana #790-2208) and 4 minutes bluing post counterstain (Ventana #760-2037). An isotype-matched mouse IgG (Ventana #760-2014) was used for negative controls.

Quantification of immune cell infiltrates in tumors

CD16+ cells were counted in fields of view under a light microscope having a grid (5x5 fields) in the eyepiece. Cells were counted in up to 25 randomly selected fields at a magnification of 400x and the density of cells, i.e. the number of cells per mm^2 of tissue area was calculated. Cells were counted in the central tumor as well as in the tissue in close proximity to the tumor cells.

Assessment of tumor response

Tumour assessment was performed by CT or MRI scan at screening and every 8 weeks beginning at cycle 4 according to modified RECIST (Response Evaluation Criteria In Solid Tumours) criteria v1.0.

Statistical considerations

Correlation of the percent change of the sum of longest diameter (SLD) from baseline to the first post-baseline tumor assessment at 8 weeks and staining of CD16+ cells in tumor biopsies obtained at baseline was evaluated with Spearman's rank correlation coefficient.

Results

Correlative analysis of baseline biomarkers with tumor response at 8 weeks (4 cycles) showed that an increased number of tumor infiltrating cells staining positive for CD16 was proportional

to a lesser increase in tumor size (r^2=-0.58, p=0.088, n=10; see Figure 1).

Example 2: Non-small cell lung cancer (NSCLC)

Study design

This was a multicenter, open-label phase Ib study of <ge-huMabEGFR> in combination with cisplatin and gemcitabine/pemetrexed in patients with advanced or recurrent EGFR-positive non-small cell lung cancer (NSCLC).

Patients were grouped into two groups according to the histology of their tumors (squamous or non-squamous), which were evaluated separately. So far, the study has been completed for the non-squamous histology group which is reported here.

Patients in this group (a total of 14 patients) were treated with <ge-huMabEGFR> in combination with cisplatin and pemetrexed according to the following dosage regimen:

Cohort 1:

- For a maximum of 6 chemotherapy cycles (18 weeks):
  Cisplatin 75 mg/m^2 i.v. on day 1 q3W (three-weekly) and pemetrexed 500 mg/m^2 i.v. on day 1 q3W for a maximum of 6 cycles.
  <ge-huMabEGFR> 1000 mg i.v. (day 1, 8) followed by 1000 mg q2W (two-weekly) i.v.
- Followed by:
  <ge-huMabEGFR> 1400 mg q2W i.v. until disease progression, unacceptable toxicity or withdrawal of consent.

Cohort 2:

- For a maximum of 6 chemotherapy cycles (18 weeks):
  Cisplatin 75 mg/m^2 i.v. on day 1 q3W and pemetrexed 500 mg/m^2 i.v. on day 1 q3W for a maximum of 6 cycles.
  <ge-huMabEGFR> 1400 mg i.v. (day 1, 8) followed by 1400 mg q2W i.v.
- Followed by:
  <ge-huMabEGFR> 1400 mg q2W i.v. until disease progression, unacceptable toxicity or withdrawal of consent.

Patient selection

Eligible patients were aged ≥18 years with an Eastern Cooperative Oncology Group performance status of ≤1 and adequate haematology, blood chemistry, renal and liver function. Patients had

histologically documented inoperable, locally advanced (stage IIIb), metastatic (stage IV) or recurrent NSCLC. Patients with prior chemotherapy or treatment with another systemic anti-cancer agent were excluded. All patients gave written informed consent and approval from local Ethics Committees was obtained. The study was conducted in accordance with Good Clinical Practice guidelines.

Administration of the drug

Infusion rate for <ge-huMabEGFR> was 10 mg/h for the first hour. Thereafter, if tolerated by the patient, the infusion rate was escalated in 30 minute intervals up to a maximum of 400 mg/h. If the first infusion was well tolerated (i.e. no serious infusion-related reactions were observed), the second infusion started at 20 mg/h for 30 minutes, followed by escalation of the infusion rate in 30 minute intervals up to 800 mg/h. Subsequent infusions started at an infusion rate of 50 mg/h, followed by escalation in 15 minute intervals up to 800 mg/h, provided the previous infusion was well tolerated by the patient. Otherwise the same infusion schedule as for the first infusion was applied.

Cisplatin and pemetrexed were administered according to local prescribing information. In general, pemetrexed should be administered at 500 mg/m^2 i.v. over 10 minutes, followed by a 30 minutes break before cisplatin administration, and cisplatin should be administered at 75 mg/m^2 i.v. over 2 hours.

Vitamin B_{12} and folic acid according to the local prescribing information were given as pre-medication pemetrexed administration (day -7 to day -1). Paracetamol, antihistamine and corticosteroids were given as premedication for the first (day -1 to day 2) and second <ge-huMabEGFR> infusion (on the day of the second infusion).

Tumor biopsies

Archival tumor specimens from diagnosis or fresh tumor biopsies taken within 21 days prior to the first administration of any study drug were collected. Biopsies were formalin-fixed and paraffin-embedded, and analysed for immune effector cell infiltrates by immunohistochemistry (IHC). Tumour-infiltrating immune effector cells were graded by counting the number of positive staining cells/mm^2. Immunohistochemical analysis and quantification of immune cell infiltrates was performed as described above (see Example 1).

Assessment of tumor response

Baseline tumor assessment was done within 21 days prior to the first administration of any study drug by chest-abdominal CT scan (or MRI). Post-baseline assessments were performed every six

weeks after baseline, i.e. on cycle 3 day 1, cycle 5 day 1 etc. until progression or withdrawal of consent.

Tumor response was evaluated according to RECIST (Response Evaluation Criteria in Solid Tumors) version 1.1 (Eisenhauer et al. (2009), Eur J Cancer 45, 228-247).

Statistical considerations

Correlation of the percent change of the sum of longest diameter (SLD) from baseline to the second post-baseline tumor assessment at 12 weeks and staining of CD16+ cells in tumor biopsies obtained at baseline was evaluated with Spearman's rank correlation coefficient.

Results

Correlative analysis of baseline biomarkers with tumor response at 12 weeks showed that an increased number of tumor infiltrating cells staining positive for CD16 was proportional to a greater decrease in tumor size (r^2=-0.52, p=0.10, n=11; see Figure 2).

Example 3: Head and neck squamous cell carcinoma (HNSCC)

Study design

This was an exploratory, open-label multicenter study to investigate the pharmakodymanics of <ge-huMabEGFR>, compared to cetuximab, in previously untreated patients with operable head and neck squamous cell carcinoma (HNSCC).

Treatments for this study were performed during the patient's waiting period before surgery (neoadjuvant setting). Patients were treated either with 700 mg <ge-huMabEGFR> on days 1 and 8, or with 400 mg cetuximab per m^2 body surface area on day 1 and 250 mg/m^2 cetuximab on day 8, followed by surgical removal of tumors 7 days after the last infusion.

Patient selection

Eligible patients were aged ≥18 years with an Eastern Cooperative Oncology Group performance status of ≤2 and adequate haematology, blood chemistry, renal and liver function. Patients had histologically confirmed squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx or larynx. Tumors had to be considered resectable with a planned surgical excision, and patients had to be naïve for chemo- and radiotherapy. All patients gave written informed consent and approval from local Ethics Committees was obtained. The study was conducted in accordance with Good Clinical Practice guidelines.

Administration of the drug

Infusion rate for <ge-huMabEGFR> was 10 mg/h for the first hour. Thereafter, if tolerated by the patient, the infusion rate was escalated in 30 minute intervals up to a maximum of 300 mg/h. If the first infusion was well tolerated (i.e. no serious infusion-related reactions were observed), subsequent infusions started at 20 mg/h for 30 minutes, followed by escalation of the infusion rate in 30 minute intervals up to 300 mg/h. Otherwise the same infusion schedule as for the first infusion was applied.

Cetuximab was administered according to local prescribing information. The recommended infusion period was 120 minutes for the first dose, and 60 minutes for the second dose. The maximum infusion rate was 10 mg/min.

For <ge-huMabEGFR> infusions, pre-medication with paracetamol, anti-histamine and corticosteroid was done, for cetuximab infusions pre-medication with anti-histamine and corticosteroid was done according to local prescribing information.

Tumor biopsies

Tumor biopsies (3-5 mm) were taken within 21 days prior to the first dosing, after the first FDG-PET/CT scan. Biopsies were formalin-fixed and paraffin-embedded, and analysed for immune effector cell infiltrates by immunohistochemistry (IHC). Tumour-infiltrating immune effector cells were graded by counting the number of positive staining cells/mm^2. Immunohistochemical analysis and quantification of immune cell infiltrates was performed as described above (see Example 1).

Assessment of tumor response

Tumors were assessed by radiologic imaging (2-^{18}F-fluoro-2-deoxy-D-glucose(FDG)-PET/CT scans) prior to any intervention (including the tumor biopsy) on days -21 to -1, as well as before surgery (not more than 3 days before surgery).

FDG-PET scans

Patients had to fast for at least 4-6 hours prior to the FDG-PET scan. Blood glucose level was checked (typically at the PET center) on the day of the FDG-PET scan and results assessed prior to the administration of FDG. The patient should have a blood glucose level $\leq$ 180 mg/dL ($\leq$ 10 mmol/mL) in order to have the FDG-PET scan. The interval between FDG administration and scanning was 60 minutes $\pm$ 10 minutes and care was taken to keep the time interval between injection and start of the scan the same at follow-up compared to baseline.

Scan Acquisition

Attenuation corrected FDG-PET scans (from skull base to mid-thigh) were performed. The patient was administered 370–740 MBq (10-20 mCi) FDG intravenously (injected activity was dependent on local practice and scanner type) (Shankar et al. (2006), J Nucl Med 47, 1059-66). The administered activity and time of FDG administration and body weight on the day of scanning was recorded for subsequent calculation of maximum tumor standardized uptake value (SUV_{max}). Approximately one hour following the administration of FDG, a whole body PET scan (base of skull to thighs) and/or a separate head and neck view was performed. Initial and follow-up scans were done the same way, taking particular care to keep the interval between FDG administration and scanning as consistent as possible.

Wherever possible, a PET/CT scanner was used (as opposed to a dedicated PET scanner) to allow for correction of the emission scan by a low dose CT transmission scan.

Scan Interpretation

The PET/CT scans were analyzed visually and interpreted and compared qualitatively by an experienced reader. A patient had to have at least two PET scans for exploratory FDG-PET response assessment. The tumor uptake was analyzed semi-quantitatively by SUVmax according to the following equation:

SUV_{max} = [maximum radiotracer concentration in tumor (kBq/mL;µCi/mL) x body weight (kg)] / injected dose (MBq; mCi)

The change in SUV_{max} was assessed. European Organization for Research and Treatment of Cancer (EORTC) response criteria were employed for evaluation of tumor response (Young et al. (1999), Eur J Cancer 35, 1773-82).

Statistical considerations

Correlation of the percent change of the SUV from baseline to the post-baseline tumor assessment at 2 weeks and staining of CD16+ cells in tumor biopsies obtained at baseline was evaluated with Spearman's rank correlation coefficient.

Results

Correlative analysis of baseline biomarkers with tumor response at 2 weeks showed that an increased number of tumor infiltrating cells staining positive for CD16 was proportional to a greater reduction in SUV_{max} in the patient group treated with <ge-huMabEGFR> (r^2=-0.57, p=0.04, n=13; see Figure 3). No correlation was observed in the patient group treated with

cetuximab (Figure 3).

Claims

1. A method of predicting the response of a cancer patient to treatment with an ADCC-enhanced antibody, comprising determining the level of CD16+ cell infiltration in the tumor of the patient prior to treatment and comparing said level of CD16+ cell infiltration to a reference level, wherein a higher level of CD16+ cell infiltration compared to the reference level is indicative for a patient who will derive clinical benefit from the treatment.

2. The method of claim 1, wherein the method is an in vitro method and said level of CD16+ cell infiltration is determined in a tumor sample taken from the patient prior to treatment.

3. The method of claims 1 or 2, wherein said reference level is a value representative of the level of CD16+ cell infiltration in tumors of a population of patients deriving no clinical benefit from the treatment.

4. The method of claim 4, wherein said reference level is determined in vitro in tumor samples taken prior to treatment from patients deriving no clinical benefit from the treatment.

5. The method of any one of claims 1 to 4, wherein the level of CD16+ cell infiltration which is indicative for a patient who will derive clinical benefit from the treatment is at least 1.2-fold, at least 1.5-fold, at least 2-fold or at least 3-fold higher than the reference level.

6. The method of any one of claims 1 to 5, wherein said cancer is selected from the group consisting of colorectal carcinoma, non-small cell lung cancer, and head and neck squamous cell carcinoma.

7. The method of any one of claims 1 to 6, wherein said ADCC-enhanced antibody is a glycoengineered antibody comprising an increased proportion of non-fucosylated oligosaccharides it its Fc region, compared to a corresponding non-glycoengineered antibody.

8. The method of any one of claims 1 to 7, wherein said ADCC-enhanced antibody is a humanized, IgG1-subclass anti-EGFR antibody comprising a) in the heavy chain variable domain a CDR1 of SEQ ID NO: 2, a CDR2 of SEQ ID NO: 3, and a CDR3 of SEQ ID NO: 4, and b) in the light chain variable domain a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6, and a CDR3 of SEQ ID NO: 7.

9. A kit for detecting the level of CD16+ cell infiltration in a tumor, the kit comprising i) one or more compounds for detecting the level of CD16+ cell infiltration, and ii) instructions for using said kit in the method according to claims 1 to 8.

10. The kit of claim 9, wherein the kit is for in vitro use and said level of CD16+ cell infiltration is detected in a tumor sample taken from a cancer patient prior to treatment with an ADCC-enhanced antibody.

11. An ADCC-enhanced antibody for use in the treatment of cancer in a patient, wherein i) the level of CD16+ cell infiltration in the tumor of the patient is determined prior to treatment, ii) the level of CD16+ cell infiltration is compared to a reference level, and iii) the ADCC-enhanced antibody is administered to a patient having a higher level of CD16+ cell infiltration compared to the reference level.

12. The ADCC-enhanced antibody of claim 11, wherein said level of CD16+ cell infiltration in the tumor is determined in vitro in a tumor sample taken from the patient prior to treatment.

13. The ADCC-enhanced antibody of claims 11 or 12, wherein said reference level is a value representative of the level of CD16+ cell infiltration in tumors of a population of patients deriving no clinical benefit from the treatment.

14. The ADCC-enhanced antibody of any one of claims 11 to 13, wherein said reference level is determined in vitro in tumor samples taken prior to treatment from patients deriving no clinical benefit from the treatment.

15. The ADCC-enhanced antibody of any one of claims 11 to 14, wherein said ADCC-enhanced antibody is administered to a patient having an at least 1.2-fold, at least 1.5-fold, at least 2-fold or at least 3-fold higher level of CD16+ cell infiltration compared to the reference level.

16. The ADCC-enhanced antibody of any one of claims 11 to 15, wherein said cancer is selected from the group consisting of colorectal carcinoma, non-small cell lung cancer, and head and neck squamous cell carcinoma.

17. The ADCC-enhanced antibody of any one of claims 11 to 16, wherein said ADCC-enhanced antibody is a glycoengineered antibody comprising an increased proportion of non-fucosylated oligosaccharides it its Fc region, compared to a corresponding non-glycoengineered antibody.

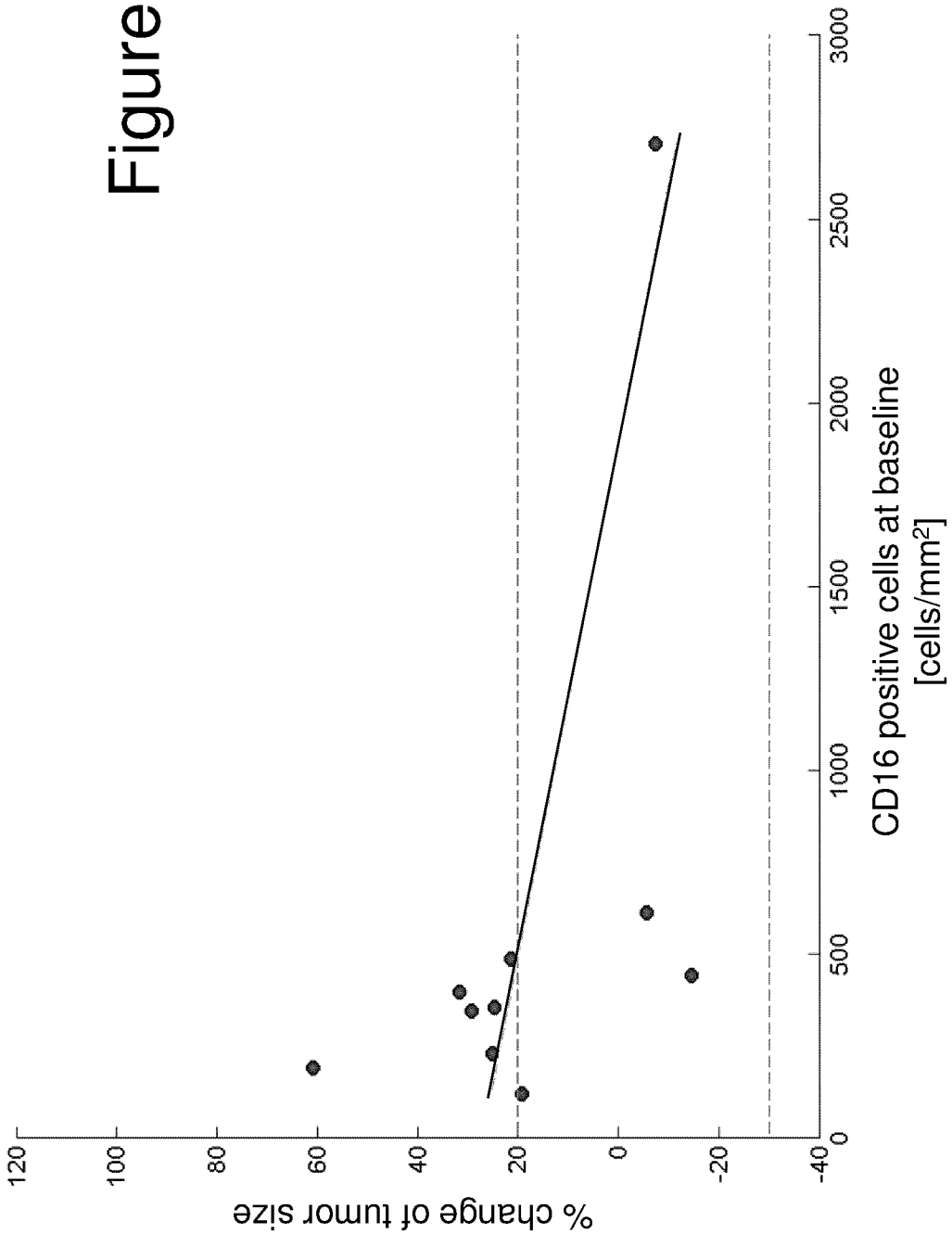
18. The ADCC-enhanced antibody of any one of claims 11 to 17, wherein said ADCC-enhanced antibody is a humanized, IgG1-subclass anti-EGFR antibody comprising a) in the heavy chain variable domain a CDR1 of SEQ ID NO: 2, a CDR2 of SEQ ID NO: 3, and a CDR3 of SEQ ID NO: 4, and b) in the light chain variable domain a CDR1 of SEQ ID NO: 5, a CDR2 of SEQ ID NO: 6, and a CDR3 of SEQ ID NO: 7.

19. A method for the treatment of cancer in a patient, wherein i) the level of CD16+ cell infiltration in the tumor of the patient is determined prior to treatment, ii) the level of CD16+ cell infiltration is compared to a reference level, and iii) an ADCC-enhanced antibody is administered to a patient having a higher level of CD16+ cell infiltration compared to the reference level.

20. A pharmaceutical composition comprising an ADCC-enhanced antibody for the treatment of a cancer patient having an increased level of CD16+ cell infiltration in the tumor prior to treatment relative to a reference level.

21. The invention as described herein.

Figure 1


% change of tumor size
120
100
80
60
40
20
0
-20
-40
0
500
1000
1500
2000
2500
3000
CD16 positive cells at baseline
[cells/mm2]

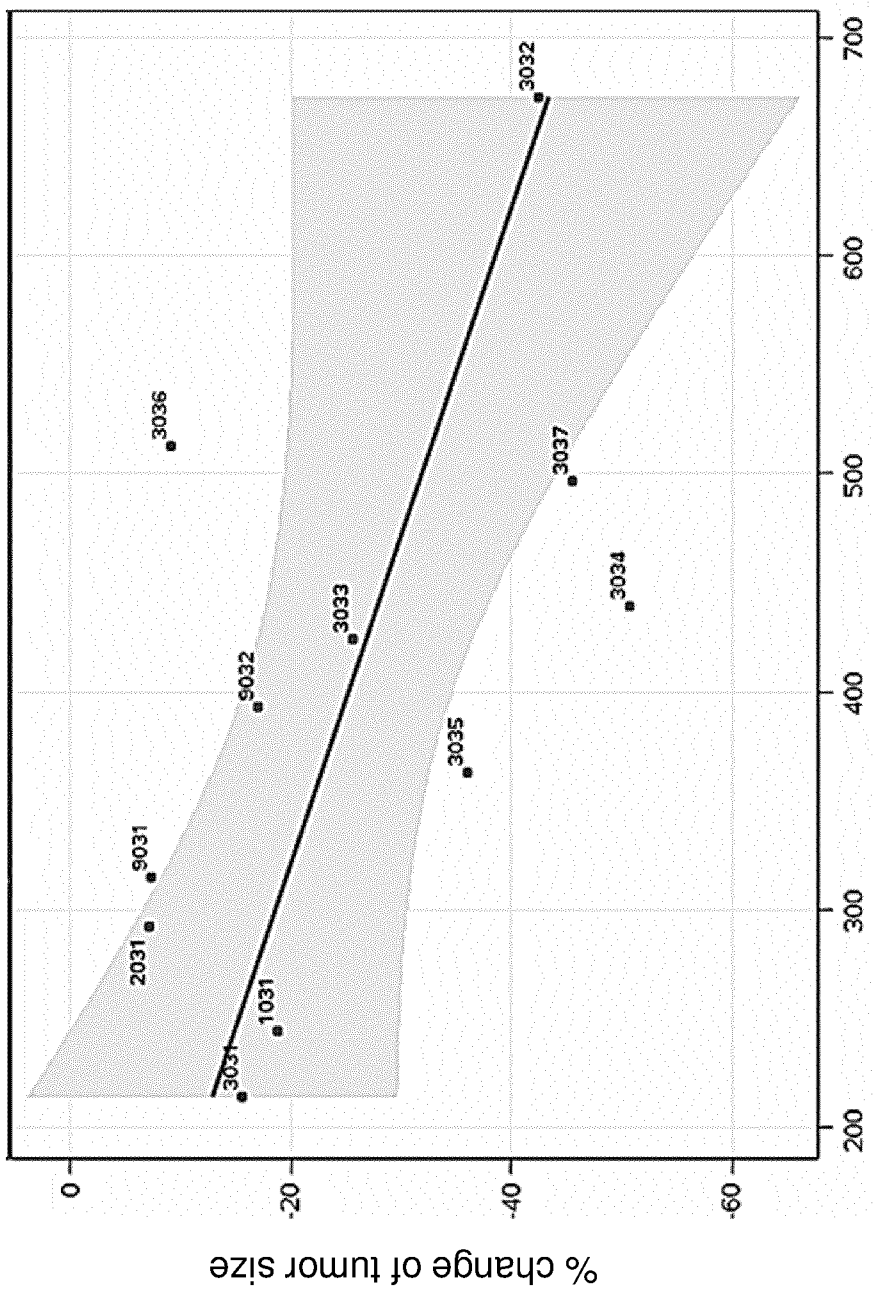

% change of tumor size
0
-20
-40
-60
200
300
400
500
600
700
CD16 positive cells at baseline
[cells/mm²]
3031
1031
2031
9031
9032
3033
3035
3037
3034
3036
3032

Figure 2

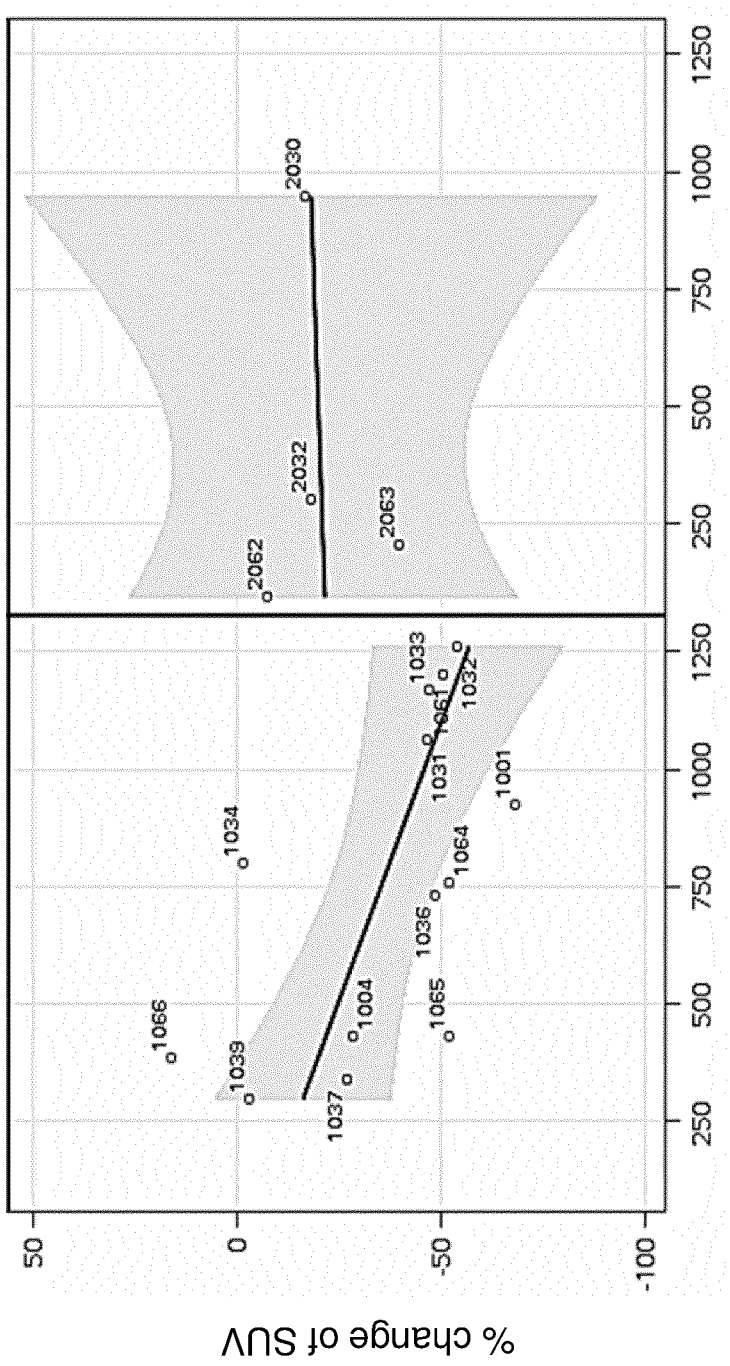

% change of SUV
50
0
-50
-100
250
500
750
1000
1250
1066
1039
1034
1037
1004
1065
1036
1064
1031
1061
1033
1032
1001
2062
2032
2063
2030
CD16 positive cells at baseline
[cells/mm²]

Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/053600

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574
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)
G01N

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, COMPENDEX, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/167315 A1 (THIBAULT GILLES [FR] ET AL) 1 July 2010 (2010-07-01) paragraphs [0009], [0010], [0017], [0022], [0050]	9,10

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 October 2012	03/12/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Thumb, Werner

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAZ-ARES LUIS G ET AL: "Phase I Pharmacokinetic and Pharmacodynamic Dose-Escalation Study of RG7160 (GA201), the First Glycoengineered Monoclonal Antibody Against the Epidermal Growth Factor Receptor, in Patients With Advanced Solid Tumors", JOURNAL OF CLINICAL ONCOLOGY, AMERICAN SOCIETY OF CLINICAL ONCOLOGY, US, vol. 29, no. 28, 1 October 2011 (2011-10-01), pages 3783-3790, XP009153323, ISSN: 0732-183X	11-18,20
Y	abstract tables 1,2 page 3786, column 1	1-8,19

Y	US 2010/247484 A1 (BARCHET HEINRICH [DE] ET AL) 30 September 2010 (2010-09-30) the whole document in particular sequences 28,29	1-21

Y	WILSON SUSAN ET AL: "IL-2 mediated NK cell expansion correlates with clinical response to rituximab: Results of two phase I trials of the combination of IL-2 and rituximab", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 98, no. 11, 16 November 2001 (2001-11-16), page 602a, XP009163930, ISSN: 0006-4971 the whole document	1-21

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Y	abstract page 720, column 1, paragraph 2	1-8, 11-21

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GIUSEPPE SCONOCCHIA ET AL: "Tumor infiltration by Fc[gamma]RIII (CD16)+ myeloid cells is associated with improved survival in patients with colorectal carcinoma", INTERNATIONAL JOURNAL OF CANCER, vol. 128, no. 11, 13 October 2010 (2010-10-13), pages 2663-2672, XP55041946, ISSN: 0020-7136, DOI: 10.1002/ijc.25609	9,10
Y	abstract page 2664, column 1, paragraph 5 - column 2, paragraph 1 page 2667, column 2, paragraph 2 - page 2668, column 2	1-8, 11-21

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Y	abstract page 11992, column 2, paragraph 2 page 1192, column 1, paragraphs 1,2 page 11993, column 2, paragraph 4 - page 11994, column 1, paragraph 1	1-8, 11-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/053600

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