(19) DANMARK

(10) **DK/EP 3625259 T3**

Patent- og
Varemærkestyrelsen

(12) Oversættelse af
europæisk patentskrift

(51) Int.Cl.: *C 07 K 16/28 (2006.01)* *A 61 P 35/00 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2024-07-08**

(80) Dato for Den Europæiske Patentmyndigheds
bekendtgørelse om meddelelse af patentet: **2024-04-17**

(86) Europæisk ansøgning nr.: **18729328.7**

(86) Europæisk indleveringsdag: **2018-05-15**

(87) Den europæiske ansøgnings publiceringsdag: **2020-03-25**

(86) International ansøgning nr.: **EP2018062473**

(87) Internationalt publikationsnr.: **WO2018210793**

(30) Prioritet: **2017-05-16 EP 17171285**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

(73) Patenthaver: **Byondis B.V., Microweg 22, 6545 CM Nijmegen, Holland**

(72) Opfinder: **VERHEIJDEN, Gijsbertus Franciscus Maria, Synthon Biopharmaceuticals B.V., Microweg 22, 6545 CM Nijmegen, Holland**
**ROUWENDAL, Gerard, Synthon Biopharmaceuticals B.V., Microweg 22, 6545 CM Nijmegen, Holland**
**ARENDS, Roland Jan, Synthon Biopharmaceuticals B.V., Microweg 22, 6545 CM Nijmegen, Holland**
**VAN DEN BERG, Timo Kars, c/o Stichting Sanquin Bloedvoorziening, Plesmanlaan 125, 1066 CX Amsterdam, Holland**
**MATLUNG, Hanke Lottie, c/o Stichting Sanquin Bloedvoorziening, Plesmanlaan 125, 1066 CX Amsterdam, Holland**
**FRANKE, Katarina, c/o Stichting Sanquin Bloedvoorziening, Plesmanlaan 125, 1066 CX Amsterdam, Holland**

(74) Fuldmægtig i Danmark: **Cosmovici Intellectual Property, Paul Cosmovici, Rue du Commerce 4, 1204 Geneva, Schweiz**

(54) Benævnelse: **ANTI-SIRPALPHA-ANTISTOFFER**

(56) Fremdragne publikationer:
**WO-A1-2013/056352**
**WO-A1-2013/150043**
**WO-A1-2014/127906**
**WO-A1-2017/068164**
**WO-A1-2018/026600**
**WO-A2-2015/138600**
**WO-A2-2017/178653**
**US-A1- 2003 054 415**
**US-A1- 2015 166 654**
**US-A1- 2016 340 397**

**Fortsættes ...**

ZHAO ET AL.: PNAS, vol. 108, no. 45, 2011, pages 18342-18347, XP055232927, cited in the application
E. M. VAN BEEK ET AL: "Signal Regulatory Proteins in the Immune System", THE JOURNAL OF IMMUNOLOGY, vol. 175, no. 12, 8 December 2005 (2005-12-08), pages 7781-7787, XP055348305, US ISSN: 0022-1767, DOI: 10.4049/jimmunol.175.12.7781
MATOZAKI T ET AL: "Functions and molecular mechanisms of the CD47-SIRPalpha signalling pathway", TRENDS IN CELL BIOLOGY, ELSEVIER SCIENCE LTD, XX, vol. 19, no. 2, 1 February 2009 (2009-02-01), pages 72-80, XP025913010, ISSN: 0962-8924, DOI: 10.1016/J.TCB.2008.12.001 [retrieved on 2009-01-12]
AKIKO HAYASHI ET AL: "Positive Regulation of Phagocytosis by SIRP[beta] and Its Signaling Mechanism in Macrophages", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 279, no. 28, 9 July 2004 (2004-07-09) , pages 29450-29460, XP055408795, ISSN: 0021-9258, DOI: 10.1074/jbc.M400950200
P.-A. OLDENBORG: "Role of CD47 as a Marker of Self on Red Blood Cells", SCIENCE, vol. 288, no. 5473, 16 June 2000 (2000-06-16), pages 2051-2054, XP55304810, US ISSN: 0036-8075, DOI: 10.1126/science.288.5473.2051
JIANG P ET AL: "INTEGRIN-ASSOCIATED PROTEIN IS A LIGAND FOR THE P84 NEURAL ADHESIONMOLECULE", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 274, no. 2, 8 January 1999 (1999-01-08), pages 559-562, XP000984770, ISSN: 0021-9258, DOI: 10.1074/JBC.274.2.559
CHUANG W ET AL: "Central nervous system antigen P84 can serve as a substrate for neurite outgrowth", DEVELOPMENTAL BIOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 137, no. 2, 1 February 1990 (1990-02-01), pages 219-232, XP024787358, ISSN: 0012-1606, DOI: 10.1016/0012-1606(90)90249-I [retrieved on 1990-02-01]
Y. LIU ET AL: "Signal Regulatory Protein (SIRPalpha ), a Cellular Ligand for CD47, Regulates Neutrophil Transmigration", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 12, 15 March 2002 (2002-03-15), pages 10028-10036, XP55030073, ISSN: 0021-9258, DOI: 10.1074/jbc.M109720200
MOTEGI SEI-ICHIRO ET AL: "Role of the CD47-SHPS-1 system in regulation of cell migration", EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, WILEY, DE, vol. 22, no. 11, 2 June 2003 (2003-06-02), pages 2634-2644, XP002481062, ISSN: 0261-4189, DOI: 10.1093/EMBOJ/CDG278
WEISKOPF KIPP ET AL: "Direct SIRPa Blockade Augments Macrophage Responses to Therapeutic Anticancer Antibodies", BLOOD, vol. 124, no. 21, 2014, page 2729, XP008179639, ISSN: 0006-4971, DOI: HTTP://WWW.BLOODJOURNAL.ORG/CONTENT/124/21 /2729 [retrieved on 2014-12-05]
HO JENNY M ET AL: "Targeting SIRP alpha in cancer", ONCOIMMUNOLOGY, LANDES BIOSCIENCE, US, vol. 2, no. 2, 31 January 2013 (2013-01-31), pages e23081-1, XP009506875, ISSN: 2162-4011, DOI: 10.4161/ONCI.23081 [retrieved on 2013-02-01]
X. W. Zhao ET AL: "CD47-signal regulatory protein-alpha (SIRPalpha) interactions form a barrier for antibody-mediated tumor cell destruction: Supporting Information", Proceedings of the National Academy of Sciences, 31 October 2011 (2011-10-31), pages 1-3, XP55132587, DOI: 10.1073/pnas.1106550108 Retrieved from the Internet: URL:http://www.pnas.org/content/suppl/2011 /10/25/1106550108.DCSupplemental/pnas.2011 06550SI.pdf#nameddest=SF3 [retrieved on 2014-07-31]
D. HATHERLEY ET AL: "The Structure of the Macrophage Signal Regulatory Protein ? (SIRP?) Inhibitory Receptor Reveals a Binding Face Reminiscent of That Used by T Cell Receptors", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 282, no. 19, 16 March 2007 (2007-03-16), pages 14567-14575, XP55081473, ISSN: 0021-9258, DOI: 10.1074/jbc.M611511200
JOANNE E NETTLESHIP ET AL: "Crystal structure of signal regulatory protein gamma (SIRP[gamma]) in complex with an antibody Fab frag", BMC STRUCTURAL BIOLOGY, BIOMED CENTRAL LTD., LONDON, GB, vol. 13, no. 1, 4 July 2013 (2013-07-04), page 13, XP021156553, ISSN: 1472-6807, DOI: 10.1186/1472-6807-13-13
LIU ET AL: "Functional Elements on SIRP@a IgV Domain Mediate Cell Surface Binding to CD47", JOURNAL OF MOLECULAR BIO, ACADEMIC PRESS, UNITED KINGDOM, vol. 365, no. 3, 23 December 2006 (2006-12-23), pages 680-693, XP005733360, ISSN: 0022-2836, DOI: 10.1016/J.JMB.2006.09.079
LEE ET AL: "Novel structural determinants on SIRP alpha that mediate binding to CD47.", THE JOURNAL OF IMMUNOLOGY, 1 January 2007 (2007-01-01), pages 7741-7750, XP55081468,

**Description**

**FIELD OF THE INVENTION**

5 **[0001]** The present invention relates to antibodies against SIRPα and the use of these antibodies in the treatment of cancer, optionally in combination with other anti-cancer therapeutics.

**BACKGROUND OF THE PRESENT INVENTION**

10 **[0002]** Since the late 1990s, therapeutic antibodies have been available for the treatment of cancer. These therapeutic antibodies can act upon malignant cells via different pathways. The signalling pathways triggered by binding of the antibody to its target on malignant cells result in inhibition of cell proliferation or in apoptosis. The Fc region of the therapeutic antibody can trigger complement dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). However, therapeutic antibodies are often not effective 15 enough as monotherapy. One option to improve the efficacy of therapeutic antibodies is through improving ADCC and/or ADCP. This has been done by improving the affinity of the Fc region for Fey receptors, e.g. by amino acid substitutions (Richards et al. Mol. Cancer Ther. 2008, 7(8), 2517-2527) or by influencing the glycosylation of the Fc region (Hayes et al. J. Inflamm. Res. 2016, 9, 209-219).

**[0003]** Another way of improving the ADCC and/or ADCP of a therapeutic antibody is by combining the therapeutic 20 antibody with an antagonistic antibody against signal regulatory protein α (anti-SIRPα) or an anti-CD47 antibody (WO2009/131453). When CD47 binds to the inhibitory immunoreceptor SIRPα expressed on monocytes, macrophages, dendritic cells and neutrophils, SIRPα transmits an inhibitory signal that prevents destruction of cancer cells by phagocytosis or other Fc-receptor-dependent cell destruction mechanisms of immune effector cells.

**[0004]** Tumour cells use up-regulation of CD47 as a mechanism to evade the anti-tumour immune response induced 25 by a therapeutic antibody. Anti-CD47 or anti-SIRPα antibodies block the inhibitory signalling generated via the CD47-SIRPα axis, resulting in an increase in ADCC and/or ADCP.

**[0005]** Most clinical research related to the CD47-SIRPα interaction has been focused on anti-CD47 antibodies, both as monotherapy and as therapy in combination with a therapeutic antibody (Weiskopf. Eur. J. Cancer 2017, 76, 100-109). Research regarding anti-CD47 antibodies as anti-cancer therapeutics is growing, despite the fact that CD47 is also 30 expressed on the surface of cells in most normal tissues.

**[0006]** Little research has been conducted on anti-cancer monotherapy or combination therapy using anti-SIRPα antibodies. The majority of the work on anti-SIRPα antibodies is mechanistic research regarding the CD47-SIRPα interaction and has been performed using murine anti-SIRPα antibodies; e.g. murine 12C4 and 1.23A increased neutrophil mediated ADCC of trastuzumab opsonised SKBR3 cells (Zhao et al. PNAS 2011, 108(45), 18342-18347). 35 WO2015/138600 discloses murine anti-human SIRPα antibody KWAR23 and its chimeric Fab fragment, which increased the *in vitro* phagocytosis of i.a. cetuximab. Humanized KWAR23 with a human IgGi Fc part comprising a N297A mutation is disclosed in WO2018/026600. WO2013/056352 discloses $IgG_4$ 29AM4-5 and other $IgG_4$ human anti-SIRPα antibodies. The $IgG_4$ 29AM4-5, dosed three times per week for four weeks at 8 mg/kg, reduced leukaemic engraftment of primary human AML cells injected into the right femur of NOD scid gamma (NSG) mice.

40 **[0007]** SIRPα is a member of the family of signal regulatory proteins (SIRP), transmembrane glycoproteins with extracellular Ig-like domains present on immune effector cells. The NH2-terminal ligand binding domain of SIRPα is highly polymorphic (Takenaka et al. Nature Immun. 2007, 8(12), 1313-1323). However, this polymorphism does not influence binding to CD47 significantly. $SIRP\alpha_{BIT}$(v1) and $SIRP\alpha_1$ (v2) are the two most common and most divergent (13 residues different) polymorphs (Hatherley et al. J. Biol. Chem. 2014, 289(14), 10024-10028). Other biochemically characterized 45 human SIRP family members are $SIRP\beta_1$, and SIRPγ.

**[0008]** $SIRP\beta_1$ does not bind CD47 (van Beek et al. J. Immunol. 2005, 175 (12), 7781-7787, 7788-7789) and at least two $SIRP\beta_1$ polymorphic variants are known, $SIRP\beta_{1v1}$ (ENSP00000371018) and $SIRP\beta_{1v2}$ (ENSP00000279477). Although the natural ligand of $SIRP\beta_1$ is yet unknown, *in vitro* studies using anti-$SIRP\beta_1$ specific antibodies show that engagement of $SIRP\beta_1$ promotes phagocytosis in macrophages by inducing the tyrosine phosphorylation of DAP12, 50 Syk, and SLP-76, and the subsequent activation of a MEK-MAPK-myosin light chain kinase cascade (Matozaki et al. J. Biol. Chem. 2004, 279(28), 29450-29460).

**[0009]** SIRPγ is expressed on T-cells and activated NK-cells and binds CD47 with a 10-fold lower affinity as compared to SIRPα. The CD47-SIRPγ interaction is involved in the contact between antigen-presenting cells and T-cells, co-stimulating T-cell activation and promoting T-cell proliferation (Piccio et al. Blood 2005, 105, 2421-2427). Furthermore, 55 CD47-SIRPγ interactions play a role in the transendothelial migration of T-cells (Stefanisakis et al. Blood 2008, 112, 1280-1289).

**[0010]** The anti-SIRPα antibodies known in the art are less suitable for use in SIRPα-directed mono- or combination therapy, because they are either not specific for human SIRPα, or they are too specific. The prior art antibodies KWAR23,

SESAS, 29AM4-5 and 12C4 are not specific, as they also bind to human SIRPγ. Binding to SIRPγ, which is expressed on T-cells, might negatively influence T-cell proliferation and recruitment. Other anti-SIRPα antibodies have a too limited specificity, e.g. 1.23A mAb only recognizes the human SIRPα polymorphic variant $SIRP\alpha_1$ and not the variant $SIRP\alpha_{BIT}$, which is predominant in at least the Caucasian population (X.W. Zhao et al. PNAS 2011, 108(45), 18342-18347).

5 **[0011]** Besides using anti-SIRPα antibodies to increase ADCC of a therapeutic antibody, these antibodies may also be used to directly target SIRPα-expressing cancer types. Anti-SIRPα antibodies comprising wild-type human -Fc may be suitable to treat cancers expressing SIRPα, such as renal cell carcinoma and malignant melanoma, as murine anti-SIRPα antibodies having a functional Fc region slowed tumour formation in mice injected with Renca cells and B16BL6 melanoma cells, both expressing SIRPα (Yanagita et al. JCI Insight 2017, 2(1), e89140).

10 **[0012]** In conclusion, a need remains for anti-SIRPα antibodies which have low binding to SIRPγ, which bind specifically to both $SIRP\alpha_1$ and $SIRP\alpha_{BIT}$ polymorphic variants and which are suitable for use in anti-cancer therapy either alone or in combination with therapeutic antibodies.

## BRIEF DESCRIPTION OF THE PRESENT INVENTION

15

**[0013]** The present invention relates to antibodies against SIRPα that are suitable for use in anti-cancer therapy. The invention further relates to the use of the antibodies in the treatment of human solid tumours and haematological malignancies.

20

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0014]**

Figure 1. Comparison of the ADCC measured in % cytotoxicity of trastuzumab (Tmab) alone, trastuzumab in combination with the murine 12C4 anti-SIRPα antibody (mu12C4), trastuzumab in combination with an antibody wherein murine 12C4 variable regions are grafted onto the human IgGi constant region (12C4huIgGi), and trastuzumab in combination with an antibody wherein murine 12C4 variable regions are grafted onto the human IgGi constant region comprising the amino acid substitutions L234A and L235A (12C4huIgGiLALA), measured on SKBR3 HER2-positive breast cancer cells using human neutrophils as effector cells.

Figure 2. Comparison of % ADCC relative to trastuzumab (set to 100%) of trastuzumab in combination with the anti-SIRPα antibodies 1-9 having a human IgGi constant region comprising the amino acid substitutions L234A and L235A, anti-SIRPα antibody 12C4huIgGiLALA (12C4LALA) and anti-CD47 antibody B6H12huIgGiLALA (B6H12LALA) on SKBR3 cells. Filled squares, (■), are the values measured with neutrophils of donors having the $SIRP\alpha_{BIT}$ variant, open circles, (○), are the values measured with neutrophils of donors having the $SIRP\alpha_1$ variant. Columns are the average of all donors; error bars represent the standard deviation.

Figure 3. Comparison of % ADCC relative to trastuzumab alone and trastuzumab in combination with the anti-SIRPα antibodies 4, 7, 10, 14 in various concentrations (dose response curves) having a human IgGi constant region comprising the amino acid substitutions L234A and L235A, and anti-SIRPα antibody 12C4huIgGiLALA (12C4LALA) on SKBR3 cells. Neutrophils of two donors (△, o) having the $SIRP\alpha_{BIT}$ variant. Columns are the average of the two donors.

Figure 4. Comparison of % ADCC relative to trastuzumab alone and trastuzumab in combination with the anti-SIRPα antibodies 4, 7, 10, 13, 14, 15 and 16 having a human IgG1 constant region comprising the amino acid substitutions L234A and L235A, and anti-SIRPα antibody 12C4huIgG1LALA (12C4LALA) on SKBR3 cells. Neutrophils of donors having the $SIRP\alpha_{BIT}$ variant (△, ⬡, ▽,◇ ), having the $SIRP\alpha_1$ variant (○, ◓) and neutrophils of a donor which variant was not determined (□) were used. Columns are the average of the donors.

## DETAILED DESCRIPTION OF THE PRESENT INVENTION

50 **[0015]** No approved therapeutics directed against SIRPα are available, although this target has been shown to play an important role in tumour immune evasion mechanisms. In addition, SIRPα is expressed on various malignant cells, rendering it a potential tumour associated antigen.

**[0016]** The present invention relates to antagonistic anti-SIRPα antibodies which exhibit specific binding to the two predominant SIRPα polymorphic variants $SIRP\alpha_{BIT}$ and $SIRP\alpha_1$, that do not bind to SIRPγ and that increase the ADCC and/or ADCP of therapeutic antibodies.

55 **[0017]** The term "antibody" as used throughout the present specification refers to a monoclonal antibody (mAb) comprising two heavy chains and two light chains. Antibodies may be of any isotype such as IgA, IgE, IgG, or IgM antibodies. Preferably, the antibody is an IgG antibody, more preferably an IgGi or $IgG_2$ antibody. The antibodies may be chimeric,

humanized or human. Preferably, the antibodies of the invention are humanized. Even more preferably, the antibody is a humanized or human IgG antibody, most preferably a humanized or human IgGi mAb. The antibody may have κ (kappa) or λ (lambda) light chains, preferably κ (kappa) light chains, i.e., a humanized or human $IgG_1$-κ antibody. The antibodies may comprise a constant region that is engineered, i.e. one or more mutations may be introduced to e.g. increase half-life, and/or increase or decrease effector function.

**[0018]** The terms "monoclonal antibody" and "mAb" as used herein refer to an antibody obtained from a population of substantially homogenous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Antibodies may be generated by immunizing animals with a mixture of peptides representing the desired antigen. B-lymphocytes are isolated and fused with myeloma cells or single B-lymphocytes are cultivated for several days in the presence of conditioned medium and feeder cells. The myeloma or B-lymphocyte supernatants containing the produced antibodies are tested to select suitable B-lymphocytes or hybridomas. Monoclonal antibodies may be prepared from suitable hybridomas by the hybridoma methodology first described by Köhler et al. Nature 1975, 256, 495-497. Alternatively, the RNA of suitable B-cells or lymphoma may be lysed, RNA may be isolated, reverse transcripted and sequenced. Antibodies may be made by recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in the art, e.g. in Clackson et al. Nature 1919, 352, 624-628 and Marks et al. J. Mol. Biol. 1991, 222, 581-597.

**[0019]** The term "antigen-binding fragment" as used throughout the present specification includes a Fab, Fab' or $F(ab')_2$ fragment, a single chain (sc) antibody, a scFv, a single domain (sd) antibody, a diabody, or a minibody.

**[0020]** In humanized antibodies, the antigen-binding complementarity determining regions (CDRs) in the variable regions (VRs) of the heavy chain (HC) and light chain (LC) are derived from antibodies from a non-human species, commonly mouse, rat or rabbit. These non-human CDRs are combined with human framework regions (FR1, FR2, FR3 and FR4) of the variable regions of the HC and LC, in such a way that the functional properties of the antibodies, such as binding affinity and specificity, are retained. Selected amino acids in the human FRs may be exchanged for the corresponding original non-human species amino acids to improve binding affinity, while retaining low immunogenicity. Alternatively, selected amino acids of the original non-human species FRs are exchanged for their corresponding human amino acids to reduce immunogenicity, while retaining the antibody's binding affinity. The thus humanized variable regions are combined with human constant regions.

**[0021]** The CDRs may be determined using the approach of Kabat (in Kabat, E.A. et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD., NIH publication no. 91-3242, pp. 662, 680, 689 (1991)), Chothia (Chothia et al., Nature 1989, 342, 877-883) or IMGT (Lefranc, The Immunologist 1999, 7, 132-136). In the context of the present invention, Eu numbering is used for indicating the positions in the heavy chain and light chain constant regions of the antibody. The expression "Eu numbering" refers to the Eu index as in Kabat, E.A. et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD., NIH publication no. 91-3242, pp. 662, 680, 689 (1991).

**[0022]** Antagonistic antibodies have affinity for a specific antigen, and binding of the antibody to its antigen inhibits the function of an agonist or inverse agonist at receptors. In the present case, binding of an antagonistic anti-SIRPα antibody to SIRPα will either prevent binding of CD47 to SIRPα or disrupt the inhibitory signal that is triggered by the CD47-SIRPα binding.

**[0023]** Antagonistic anti-SIRPα antibodies may bind to the same site where CD47 binds, preventing ligation of SIRPα by CD47 and consequently inhibiting the signalling that negatively regulates the Fc-receptor-dependent action of immune effector cells. Antagonistic anti-SIRPα antibodies may also bind to a site of SIRPα that is different from the binding site of CD47, i.e. an allosteric site, and inhibit the inhibitory signalling of SIRPα without direct interference with the physical CD47-SIRPα interaction, e.g. a change in the three-dimensional shape of SIRPα. This change in the three-dimensional shape prevents (downstream) signalling upon binding to CD47. When SIRPα is bound at an allosteric site, CD47 may still be bound by SIRPα, which might cause CD47 to be less available for binding to thrombospondin-1 (TSP-1). Ligation of TSP-1 to CD47 plays a role in e.g. negative regulation of T-cell activation (Soto-Pantoja et al. Crit. Rev. Biochem. Mol. Biol. 2015, 50(3), 212-230).

**[0024]** The term "binding affinity" as used throughout the present specification, refers to the dissociation constant ($K_D$) of a particular antigen-antibody interaction. The $K_D$ is the ratio of the rate of dissociation ($k_{off}$) to the association rate ($k_{on}$). Consequently, $K_D$ equals $k_{off}/k_{on}$ and is expressed as a molar concentration (M). It follows that the smaller the $K_D$, the stronger the affinity of binding. Typically, $K_D$ values are determined by using surface plasmon resonance (SPR), typically using a biosensor system (e.g. Biacore®) using methods known in the art (e.g. E.S. Day et al. Anal. Biochem. 2013, 440, 96-107).The term "binding affinity" may also refer to the concentration of antibody that gives half-maximal binding ($EC_{50}$) determined with e.g. an ELISA assay or as determined by flow cytometry.

**[0025]** The term "specific binding" as used throughout the present specification relates to binding between an antibody and its antigen with a $K_D$ of typically less than $10^{-7}$ M, such as $10^{-8}$ M, $10^{-9}$ M, $10^{-10}$ M, $10^{-11}$ M or even lower as determined by SPR at 25°C.

**[0026]** The term "low affinity" as used throughout the present specification is interchangeable with the phrases "does/do not bind" or "is/are not binding to", and refers to a binding affinity between an antibody and its antigen with an $EC_{50}$ larger than 1500 ng/ml as determined using an ELISA assay, or where no specific binding is observed between the immobilized antigen and the antibody as determined by SPR.

**[0027]** The term "high affinity" as used throughout the present specification and refers to a binding affinity between an antibody and its antigen with a $K_D$ of typically less than $10^{-10}$ M, $10^{-11}$ M or even lower as determined by SPR at 25°C.

**[0028]** The present invention relates to an anti-SIRPα antibody or an antigen-binding fragment thereof, comprising heavy chain (HC) and light chain (LC) variable region (VR) complementarity determining regions (CDRs) CDR1, CDR2 and CDR3, wherein:

a. HC VR CDR1 consists of the amino acid sequence HGIS,
b. HC VR CDR2 consists of the amino acid sequence TIGTGVITYFASWAKG,
c. HC VR CDR3 consists of the amino acid sequence GSAWNDPFDP,
d. LC VR CDR1 consists of the amino acid sequence QASQSVYGNNDLA,
e. LC VR CDR2 consists of the amino acid sequence LASTLAT, and
f. LC VR CDR3 consists of the amino acid sequence LGGGDDEADNV,

wherein the CDRs are determined according to Kabat numbering.

**[0029]** The present invention relates to an anti-SIRPα antibody or an antigen-binding fragment thereof comprising heavy chain (HC) and light chain (LC) variable region (VR) complementarity determining regions (CDRs) CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 13 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 14.

**[0030]** Disclosed but do not part of the claimed invention is an anti-SIRPα antibody or an antigen-binding fragment thereof comprising heavy chain (HC) and light chain (LC) variable region (VR) complementarity determining regions (CDRs) selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2;
b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:3 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:4;
c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6;
d. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:7 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:8;
e. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:9 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 10;
f. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 12;
g. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 15 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 16; and
h. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 17 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 18, wherein the CDRs are determined according to Kabat numbering.

**[0031]** In a preferred embodiment, the present invention relates to an anti-SIRPα antibody according to the invention or an antigen-binding fragment thereof as defined hereinabove, wherein the antibody shows specific binding to both $SIRP\alpha_{BIT}$ and $SIRP\alpha_1$ and does not bind to SIRPγ.

**[0032]** In a more preferred embodiment, the anti-SIRPα antibody or an antigen-binding fragment thereof specifically binds $SIRP\alpha_{BIT}$ with a $K_D$ below $10^{-9}$ M and binds $SIRP\alpha_1$ with a $K_D$ below $10^{-7}$ M, wherein the $K_D$ is measured with SPR at 25°C. Preferably, the anti-SIRPα antibody or an antigen-binding fragment thereof binds $SIRP\alpha_1$ with a $K_D$ below $10^{-8}$ M.

**[0033]** In another more preferred embodiment, the anti-SIRPα antibody or an antigen-binding fragment thereof specifically binds $SIRP\alpha_{BIT}$ and $SIRP\alpha_1$ with a $K_D$ below $10^{-9}$ M, wherein the $K_D$ is measured with SPR at 25°C.

**[0034]** In an even more preferred embodiment, the anti-SIRPα antibody or an antigen-binding fragment thereof specifically binds $SIRP\alpha_{BIT}$ and $SIRP\alpha_1$ with a $K_D$ below $10^{-10}$ M. Preferably, the anti-SIRPα or an antigen-binding fragment thereof antibody specifically binds $SIRP\alpha_{BIT}$ with a $K_D$ below $10^{-10}$ M and $SIRP\alpha_1$ with a $K_D$ below $10^{-11}$ M. Typically, the anti-SIRPα antibody according to the invention as defined hereinabove is a chimeric, humanized or human antibody. Preferably, the anti-SIRPα antibody is a humanized or human antibody. More preferably, the anti-SIRPα antibody is a humanized antibody. In a particular embodiment, the humanized anti-SIRPα antibody or an antigen-binding fragment thereof according to the invention comprises a HCVR and a LCVR selected from the group consisting of:

a. HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:36;
b. HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:37;
c. HCVR amino acid sequence of SEQ ID NO: 13 and LCVR amino acid sequence of SEQ ID NO:38; and
d. HCVR amino acid sequence of SEQ ID NO: 13 and LCVR amino acid sequence of SEQ ID NO:37.

**[0035]** In yet another preferred embodiment, the humanized anti-SIRP$\alpha$ antibody or an antigen-binding fragment thereof comprises HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:36.

**[0036]** In yet another preferred embodiment, the humanized anti-SIRP$\alpha$ antibody or an antigen-binding fragment thereof comprises HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:37.

**[0037]** In yet another preferred embodiment, the humanized anti-SIRP$\alpha$ antibody or an antigen-binding fragment thereof comprises HCVR amino acid sequence of SEQ ID NO: 13 and LCVR amino acid sequence of SEQ ID NO:38.

**[0038]** In yet another preferred embodiment, the humanized anti-SIRP$\alpha$ antibody or an antigen-binding fragment thereof comprises HCVR amino acid sequence of SEQ ID NO: 13 and LCVR amino acid sequence of SEQ ID NO:37.

**[0039]** Disclosed but not part of the claimed invention is the humanized anti-SIRP$\alpha$ antibody or an antigen-binding fragment thereof comprising HCVR amino acid sequence of SEQ ID NO:30 and LCVR amino acid sequence of SEQ ID NO:31.

**[0040]** Disclosed but not part of the claimed invention is the humanized anti-SIRP$\alpha$ antibody or an antigen-binding fragment thereof comprising HCVR amino acid sequence of SEQ ID NO:32 and LCVR amino acid sequence of SEQ ID NO:33.

**[0041]** Disclosed but not part of the claimed invention is the humanized anti-SIRP$\alpha$ antibody or an antigen-binding fragment thereof comprising HCVR amino acid sequence of SEQ ID NO:34 and LCVR amino acid sequence of SEQ ID NO:8.

**[0042]** Besides binding to both human (hu)SIRP$\alpha_{BIT}$ and (hu)SIRP$\alpha_1$, the antibodies according to the invention may also bind to cynomolgus monkey (cy)SIRP$\alpha$, enabling *in vivo* studies in a relevant animal model.

**[0043]** The antibodies according to the invention may bind to a site of SIRP$\alpha$ that is different from the binding site of CD47, i.e. an allosteric site and inhibit the inhibitory signalling of SIRP$\alpha$ without direct interference with the physical CD47-SIRP$\alpha$ interaction. Alternatively, the antibodies may bind to the same site where CD47 binds, preventing ligation of SIRP$\alpha$ by CD47 and consequently inhibiting the signalling that negatively regulates the Fc-receptor-dependent action of immune effector cells.

**[0044]** The anti-SIRP$\alpha$ antibodies according to the invention or antigen-binding fragments thereof as described hereinabove are more specific than known anti-SIRP$\alpha$ antibodies, and show excellent affinity for both SIRP$\alpha_{BIT}$ and SIRP$\alpha_1$. As well, the anti-SIRP$\alpha$ antibodies according to the invention do not bind to SIRP$\gamma$.

**[0045]** In one particular embodiment, the anti-SIRP$\alpha$ antibody according to the invention comprises an Fc region that binds to activating Fc receptors present on human immune effector cells. Such anti-SIRP$\alpha$ antibody is suitable for monotherapy of SIRP$\alpha$-positive human solid tumours and haematological malignancies as it can induce ADCC and/or ADCP. Human immune effector cells possess a variety of activating Fc receptors, which upon ligation trigger phagocytosis, cytokine release, ADCC and/or ADCP, etc. Examples of these receptors are Fcy receptors, e.g. Fc$\gamma$RI (CD64), FcyRIIA (CD32), Fc$\gamma$RIIIA (CD16a), FcyRIIIB (CD16b), FcyRIIC and the Fc$\alpha$ receptor Fc$\alpha$RI (CD89). The various natural antibody isotypes bind to these receptors. E.g. IgGi binds to Fc$\gamma$RI, FcyRIIA, FcyRIIC, Fc$\gamma$RIIIA, FcyRIIIB; IgG$_2$ binds to FcyRIIA, FcyRIIC, Fc$\gamma$RIIIA; IgG$_3$ binds to Fc$\gamma$RI, FcyRIIA, FcyRIIC, FcyRIIIA, FcyRIIIB; IgG$_4$ binds to Fc$\gamma$RI, FcyRIIA, FcyRIIC, Fc$\gamma$RIIIA; and IgA binds to Fc$\alpha$RI.

**[0046]** In a preferred embodiment, the anti-SIRP$\alpha$ antibody according to the invention comprises an Fc region of the IgA or IgG isotype. More preferred is an anti-SIRP$\alpha$ antibody comprising an Fc region of the IgGi, IgG$_2$, IgG$_3$ or IgG$_4$ isotype; the IgGi, IgG$_2$ or IgG$_4$ isotype is even more preferred. Most preferred is an anti-SIRP$\alpha$ antibody comprising an Fc region of the IgGi isotype.

**[0047]** Although the anti-SIRP$\alpha$ antibodies comprising an Fc region that binds to activating Fc receptors present on human immune effector cells may be suitable to treat cancers expressing SIRP$\alpha$, chimeric anti-SIRP$\alpha$ IgGi antibodies did not show the expected results when tested *in vitro* in combination with other antibodies that comprise a human Fc region that binds to activating Fc receptors present on human immune effector cells (i.e. antibodies that are able to induce ADCC and/or ADCP). Results of *in vitro* ADCC assays showed that a chimeric IgGi anti-SIRP$\alpha$ antibody does not increase the ADCC of such other antibody as much as expected on the basis of earlier results using murine antibodies.

**[0048]** Therefore, the invention relates to anti-SIRP$\alpha$ antibodies that exhibit reduced binding to or low affinity for activating Fc receptors present on human immune effector cells. Such anti-SIRP$\alpha$ antibodies comprise a modified Fc region in which one or more amino acids have been substituted by (an)other amino acid(s) when compared to a similar unmodified Fc region. Reduced binding means that the affinity of the anti-SIRP$\alpha$ antibody comprising a modified Fc region for the activating Fc receptors is less than the affinity of an anti-SIRP$\alpha$ antibody with the same variable regions comprising a similar unmodified Fc region. The binding affinity of antibodies for activating Fc receptors is typically measured using Surface Plasmon Resonance (SPR) or flow cytometry using methods known in the art, e.g. the method

of Harrison et al. in J. Pharm. Biomed. Anal. 2012, 63, 23-28. Antibodies exhibiting reduced binding to or low affinity for the human Fcα or Fcγ receptor in combination with a therapeutic antibody are especially effective in cellular destruction of cancer cells by increasing ADCC and/or ADCP of effector immune effector cells. Typically, the Fc region of the anti-SIRPα antibody according to the invention is modified to reduce binding to activating Fc receptors present on human immune effector cells.

**[0049]** Therefore, the anti-SIRPα antibody according to the invention comprises a modified Fc region that exhibits reduced binding to or low affinity for a human Fcα or Fcγ receptor. For instance, the IgGi binding to an Fcγ receptor can be reduced by substituting one or more IgGi amino acids selected from the group consisting of L234, L235, G237, D265, D270, N297, A327, P328, and P329 (Eu numbering); the $IgG_2$ binding can be reduced by introducing e.g. one or more of the following amino acid substitutions V234A, G237A, P238S, H268A, V309L, A330S, and P331S; or H268Q, V309L, A330S, and P331S (numbering analogue to IgGi Eu numbering) (Vafa et al. Methods 2014, 65, 114-126); the $IgG_3$ binding can be reduced by introducing e.g. the amino acid substitutions L234A and L235A, or the amino acid substitutions L234A, L235A and P331S (Leoh et al. Mol. Immunol. 2015, 67, 407-415); and the $IgG_4$ binding can be reduced by introducing e.g. the amino acid substitutions S228P, F234A and L235A ((numbering analogue to IgGi Eu numbering) (Parekh et al. mAbs 2012, 4(3), 310-318). IgA binding to the Fcα receptor can be reduced by introducing e.g. one or more of the amino acid substitutions L257R, P440A, A442R, F443R, and P440R (sequential numbering, Pleass et al. J. Biol. Chem. 1999, 271(33), 23508-23514).

**[0050]** Preferably, the anti-SIRPα antibody according to the invention comprises a modified Fc region that exhibits reduced binding to or low affinity for a human Fcγ receptor. More preferably, the modified Fc region is an Fc region of the IgG isotype. Even more preferably, the modified Fc region is an Fc region of the IgGi, $IgG_2$ or $IgG_4$ isotype.

**[0051]** In a preferred embodiment, the anti-SIRPα antibody according to the invention comprises a modified human IgGi Fc region comprising one or more amino acid substitutions at one or more positions selected from the group consisting of L234, L235, G237, D265, D270, N297, A327, P328, and P329 (Eu numbering).

**[0052]** Preferably, the anti-SIRPα antibody comprises a modified Fc IgGi region, which does not comprise either amino acid substitution N297A or N297G. More preferably, the anti-SIRPα antibody comprises a modified Fc IgGi region, which does not comprise an amino acid substitution at position N297.

**[0053]** In one embodiment, the modified human IgGi Fc region comprises one or more amino acid substitutions selected from the group consisting of L234A, L234E, L235A, G237A, D265A, D265E, D265N, D270A, D270E, D270N, N297A, N297G, A327Q, P328A, P329A and P329G. Preferably, the one or more amino acid substitutions are selected from the group consisting of L234A, L234E, L235A, G237A, D265A, D265E, D265N, N297A, P328A, P329A and P329G.

**[0054]** In another embodiment, the modified human IgGi Fc region comprises one or more amino acid substitutions selected from the group consisting of L234A, L234E, L235A, G237A, D265A, D265E, D265N, D270A, D270E, D270N, A327Q, P328A, P329A and P329G. Preferably, the one or more amino acid substitutions are selected from the group consisting of L234A, L234E, L235A, G237A, D265A, D265E, D265N, P328A, P329A and P329G. More preferably, the modified Fc IgGi region does not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgGi region does not comprise an amino acid substitution at position N297.

**[0055]** In a preferred embodiment, the modified human IgGi Fc region comprises the amino acid substitutions L234A and L235A, L234E and L235A, L234A, L235A and P329A or L234A, L235A and P329G. Preferably, the modified Fc IgGi region does not comprise either amino acid substitution N297A or N297G. More preferably, the modified Fc IgGi region does not comprise an amino acid substitution at position N297.

**[0056]** In another preferred embodiment, the anti-SIRPα antibody according to the invention comprises a modified human IgGi Fc region comprising the amino acid substitutions L234A and L235A or L234E and L235A, preferably amino acid substitutions L234A and L235A. More preferably, the modified Fc IgGi region does not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgGi region does not comprise an amino acid substitution at position N297.

**[0057]** The present invention further relates to a pharmaceutical composition comprising an anti-SIRPα antibody according to the invention as described hereinabove and one or more pharmaceutically acceptable excipients. Typical pharmaceutical formulations of therapeutic proteins such as antibodies take the form of lyophilized cakes (lyophilized powders), which require (aqueous) dissolution (i.e. reconstitution) before intravenous infusion, or frozen (aqueous) solutions, which require thawing before use.

**[0058]** Typically, the pharmaceutical composition is provided in the form of a lyophilized cake. Suitable pharmaceutically acceptable excipients for inclusion into the pharmaceutical composition (before freeze-drying) in accordance with the present invention include buffer solutions (e.g. citrate, histidine or succinate containing salts in water), lyoprotectants (e.g. sucrose, trehalose), tonicity modifiers (e.g. sodium chloride), surfactants (e.g. polysorbate), and bulking agents (e.g. mannitol, glycine). Excipients used for freeze-dried protein formulations are selected for their ability to prevent protein denaturation during the freeze-drying process as well as during storage.

**[0059]** The present invention further relates to an anti-SIRPα antibody according to the invention or pharmaceutical composition as described hereinabove for use as a medicament.

**[0060]** In one embodiment, the present invention relates to an anti-SIRPα antibody according to the invention or pharmaceutical composition as described hereinabove for use in the treatment of human solid tumours and haematological malignancies. The anti-SIRPα antibodies of the invention may be used in the treatment of solid tumours, such as breast cancer, renal cancer, or melanoma, or haematological malignancies, such as Acute Myeloid Leukaemia (AML).

**[0061]** In a second embodiment, the invention relates to an anti-SIRPα antibody comprising an Fc region that binds to activating Fc receptors present on human immune effector cells for use in the treatment of SIRPα-positive human solid tumours and haematological malignancies. Preferably, the Fc region that binds to activating Fc receptors present on human immune effector cells is of the IgA or IgG isotype. More preferred is an anti-SIRPα antibody comprising an Fc region of the IgGi, $IgG_2$, $IgG_3$ or $IgG_4$ isotype; the IgGi, $IgG_2$ or $IgG_4$ isotype is even more preferred. Most preferred is an anti-SIRPα antibody comprising an Fc region of the IgGi isotype for use in the treatment of SIRPα-positive human solid tumours and haematological malignancies.

**[0062]** In a third embodiment, the present invention relates to an anti-SIRPα antibody according to the invention or pharmaceutical composition as described hereinabove for use in the treatment of human solid tumours and haematological malignancies in combination with the use of one or more other anti-cancer therapies. Suitable anti-cancer therapies are surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and immunotherapy. The anti-SIRPα antibody according to the invention or pharmaceutical composition as described hereinabove may be for concomitant or sequential use in the treatment of human solid tumours and haematological malignancies in combination with the use of one or more other anti-cancer therapies. In particular, the anti-SIRPα antibody according to the invention or pharmaceutical composition as described hereinabove may be for use in the treatment of human solid tumours and haematological malignancies after the use of one or more other anti-cancer therapies.

**[0063]** Preferably, the present invention relates to an anti-SIRPα antibody according to the invention or pharmaceutical composition as described hereinabove for use in the treatment of human solid tumours and haematological malignancies in combination with the use of one or more other anti-cancer therapeutics. In particular, the anti-SIRPα antibody according to the invention or pharmaceutical composition as described hereinabove may be for use in the treatment of human solid tumours and haematological malignancies after the use of one or more other anti-cancer therapeutics.

**[0064]** Suitable anti-cancer therapeutics include chemotherapeutics, radiation therapeutics, hormonal therapeutics, targeted therapeutics and immunotherapeutic agents. Suitable chemotherapeutics include alkylating agents, such as nitrogen mustards, nitrosoureas, tetrazines and aziridines; anti metabolites, such as anti-folates, fluoropyrimidines, deoxynucleoside analogues and thiopurines; anti-microtubule agents, such as vinca alkaloids and taxanes; topoisomerase I and II inhibitors; and cytotoxic antibiotics, such as anthracyclines and bleomycins.

**[0065]** Suitable radiation therapeutics include radio isotopes, such as $^{131}$I-metaiodobenzylguanidine (MIBG), $^{32}$P as sodium phosphate, $^{223}$Ra chloride, $^{89}$Sr chloride and $^{153}$Sm diamine tetramethylene phosphonate (EDTMP).

**[0066]** Suitable agents to be used as hormonal therapeutics include inhibitors of hormone synthesis, such as aromatase inhibitors and GnRH analogues; and hormone receptor antagonists, such as selective oestrogen receptor modulators and antiandrogens.

**[0067]** Targeted therapeutics are therapeutics that interfere with specific proteins involved in tumorigenesis and proliferation and may be small molecule drugs; proteins, such as therapeutic antibodies; peptides and peptide derivatives; or protein-small molecule hybrids, such as antibody-drug conjugates. Examples of targeted small molecule drugs include mTor inhibitors, such as everolimus, temsirolimus and rapamycin; kinase inhibitors, such as imatinib, dasatinib and nilotinib; VEGF inhibitors, such as sorafenib and regorafenib; and EGFR/HER2 inhibitors such as gefitinib, lapatinib and erlotinib. Examples of peptide or peptide derivative targeted therapeutics include proteasome inhibitors, such as bortezomib and carfilzomib.

**[0068]** Immunotherapeutic agents include agents that induce, enhance or suppress an immune response, such as cytokines (IL-2 and IFN-α); immuno modulatory imide drugs, such as thalidomide, lenalidomide and pomalidomide; therapeutic cancer vaccines, such as talimogene laherparepvec; cell based immunotherapeutic agents, such as dendritic cell vaccines, adoptive T-cells and chimeric antigen receptor-modified T-cells); and therapeutic antibodies that can trigger ADCC/ADCP or CDC via their Fc region when binding to membrane bound ligands on a cancer cell.

**[0069]** Preferably, the invention relates to an anti-SIRPα antibody according to the invention or pharmaceutical composition as described hereinabove for use in the treatment of human solid tumours and haematological malignancies in combination with one or more other anti-cancer therapeutics, wherein the anti-cancer therapeutic is a targeted therapeutic or an immunotherapeutic agent. A preferred targeted therapeutic in accordance with the invention is a therapeutic antibody or an antibody-drug conjugate (ADC). The most preferred targeted therapeutic is a therapeutic antibody.

**[0070]** The term "therapeutic antibody" as used throughout the present specification refers to an antibody according to the invention or an antigen-binding fragment thereof as defined hereinabove, which is suitable for human therapy. Antibodies suitable for human therapy are of sufficient quality, safe and efficacious for treatment of specific human diseases. Quality may be assessed using the established guidelines for Good Manufacturing Practice; safety and efficacy are typically assessed using established guidelines of medicines regulatory authorities, e.g. the European Medicines Agency (EMA) or the United States Food and Drug Administration (FDA). These guidelines are well-known in the art.

**[0071]** Preferably, the therapeutic antibody is an antibody approved by a medicines regulatory authority, such as the EMA or FDA. Online databases of most Regulatory Authorities can be consulted to find whether an antibody is approved.

**[0072]** The term "ADC" as used throughout the present specification refers to a cytotoxic drug conjugated to an antibody according to the invention or an antigen-binding fragment thereof as defined hereinabove via a linker. Typically, the cytotoxic drugs are highly potent, e.g. a duocarmycin, calicheamicin, pyrrolobenzodiazepine (PBD) dimer, maytansinoid or auristatin derivative. The linker may be cleavable, e.g. comprising the cleavable dipeptide valine-citrulline (vc) or valine-alanine (va), or non-cleavable, e.g. succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC).

**[0073]** Typically, the therapeutic antibody for use in combination with an anti-SIRPα antibody according to the invention is a monospecific or bispecific antibody or antibody fragment comprising at least one HCVR and LCVR binding to a target selected from the group consisting of annexin Al, B7H3, B7H4, CA6, CA9, CA15-3, CA19-9, CA27-29, CA125, CA242, CCR2, CCRS, CD2, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD44, CD47, CD56, CD70, CD74, CD79, CD115, CD123, CD138, CD203c, CD303, CD333, CEA, CEACAM, CLCA-1, CLL-1, c-MET, Cripto, CTLA-4, DLL3, EGFL, EGFR, EPCAM, EPh (e.g. EphA2 or EPhB3), endothelin B receptor (ETBR), FAP, FcRL5 (CD307), FGF, FGFR (e.g. FGFR3), FOLR1, GCC, GPNMB, HER2, HMW-MAA, integrin α (e.g. αvβ3 and αvβ5), IGF1R, TM4SF1 (or L6 antigen), Lewis A like carbohydrate, Lewis X, Lewis Y, LIV1, mesothelin, MUC1, MUC16, NaPi2b, Nectin-4, PD-1, PD-L1, PSMA, PTK7, SLC44A4, STEAP-1, ST4 antigen (or TPBG, trophoblast glycoprotein), TF (tissue factor), Thomsen-Friedenreich antigen (TF-Ag), Tag72, TNF, TNFR, TROP2, VEGF, VEGFR, and VLA.

**[0074]** Preferred is a monospecific therapeutic antibody. More preferred is an antibody against a membrane-bound target on the surface of tumour cells.

**[0075]** Suitable therapeutic antibodies for use in combination with an anti-SIRPα antibody according to the invention include alemtuzumab, bevacizumab, cetuximab, panitumumab, rituximab, and trastuzumab.

**[0076]** Suitable ADCs for use in combination with an anti-SIRPα antibody according to the invention include trastuzumab emtansine and brentuximab vedotin.

**[0077]** In a preferred embodiment, the present invention relates to an anti-SIRPα antibody according to the invention as described hereinabove for the aforementioned use in combination with a therapeutic antibody against a membrane-bound target on the surface of tumour cells which comprises a human Fc region that binds to activating Fc receptors present on human immune effector cells.

**[0078]** Via binding to these activating Fc receptors, described hereinabove, a therapeutic antibody comprising a human Fc region that binds to activating Fc receptors present on human immune effector cells can induce ADCC and/or ADCP. Therapeutic antibodies of the human IgG, IgE, or IgA isotype comprise a human Fc region that binds to activating Fc receptors present on human immune effector cells.

**[0079]** A preferred therapeutic antibody for use according to the invention is a therapeutic antibody of the IgG or IgA isotype. More preferred is a therapeutic antibody of the IgG isotype, such as IgGi, $IgG_2$, $IgG_3$, and $IgG_4$ antibodies. Even more preferred is a therapeutic antibody of the IgGi or $IgG_2$ isotype. Most preferred is a therapeutic antibody of the IgGi isotype.

**[0080]** Preferably, the present invention relates to a humanized anti-SIRPα antibody comprising CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 13 and CDR1,

**[0081]** CDR2 and CDR3 amino acid sequences of SEQ ID NO: 14 for use in the treatment of human solid tumours and haematological malignancies in combination with the use of a therapeutic antibody against a membrane-bound target on the surface of tumour cells, which comprises a human Fc region that binds to activating Fc receptors present on human immune effector cells, wherein the anti-SIRPα antibody comprises a modified Fc region that exhibits reduced binding to a human Fcα or Fcy receptor, when compared to the same anti-SIRPα antibody comprising a wild-type Fc region.

**[0082]** Disclosed but not part of the claimed invention is a humanized anti-SIRPα antibody comprising HCVR and LCVR CDRs selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2;

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:3 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:4;

c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6;

d. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:7 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:8;

e. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:9 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 10;

f. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 12;

g. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 15 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 16; and h. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 17 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 18,

5 for use in the treatment of human solid tumours and haematological malignancies in combination with the use of a therapeutic antibody against a membrane-bound target on the surface of tumour cells, which comprises a human Fc region that binds to activating Fc receptors present on human immune effector cells, wherein the anti-SIRPα antibody comprises a modified Fc region that exhibits reduced binding to a human Fcα or Fcy receptor, when compared to the same anti-SIRPα antibody comprising a wild-type Fc region.

10 **[0083]** In a preferred embodiment, the humanized anti-SIRPα antibody for use in the treatment of human solid tumours and haematological malignancies in combination with the therapeutic antibody, comprises a modified human IgGi Fc region comprising one or more amino acid substitutions at one or more positions selected from the group consisting of L234, L235, G237, D265, D270, N297, A327, P328, and P329 (Eu numbering).

**[0084]** Preferably, the humanized anti-SIRPα antibody for use in the treatment of human solid tumours and haema-15 tological malignancies in combination with the therapeutic antibody comprises a modified Fc IgGi region, which does not comprise either amino acid substitution N297A or N297G. More preferably, the anti-SIRPα antibody comprises a modified Fc IgGi region, which does not comprise an amino acid substitution at position N297.

**[0085]** In one embodiment, the modified human IgGi Fc region comprises one or more amino acid substitutions selected from the group consisting of L234A, L234E, L235A, G237A, D265A, D265E, D265N, D270A, D270E, D270N, N297A, 20 N297G, A327Q, P328A, P329A, and P329G.

**[0086]** In another embodiment, the humanized anti-SIRPα antibody for use in the treatment of human solid tumours and haematological malignancies in combination with the therapeutic antibody comprises a modified Fc IgGi region comprising one or more amino acid substitutions selected from the group consisting of L234A, L234E, L235A, G237A, D265A, D265E, D265N, D270A, D270E, D270N, A327Q, P328A, P329A and P329G. Preferably, the one or more amino 25 acid substitutions are selected from the group consisting of L234A, L234E, L235A, G237A, D265A, D265E, D265N, P328A, P329A and P329G. More preferably, the modified Fc IgGi region does not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgGi region does not comprise an amino acid substitution at position N297.

**[0087]** In a preferred embodiment, the modified human IgGi Fc region comprises the amino acid substitutions L234A 30 and L235A, L234E and L235A, L234A, L235A and P329A or L234A, L235A and P329G. Preferably, the modified Fc IgGi region does not comprise either amino acid substitution N297A or N297G. More preferably, the modified Fc IgGi region does not comprise an amino acid substitution at position N297.

**[0088]** In another preferred embodiment, he humanized anti-SIRPα antibody for use in the treatment of human solid tumours and haematological malignancies in combination with the therapeutic antibody comprises a modified human 35 IgGi Fc region comprising the amino acid substitutions L234A and L235A or L234E and L235A, preferably amino acid substitutions L234A and L235A. More preferably, the modified Fc IgGi region does not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgGi region does not comprise an amino acid substitution at position N297.

**[0089]** In a preferred embodiment, the humanized anti-SIRPα antibody for use in the treatment of human solid tumours 40 and haematological malignancies in combination with the use of a therapeutic antibody against a membrane-bound target on the surface of tumour cells which comprises a human Fc region that binds to activating Fc receptors present on human immune effector cells, comprises an Fc region comprising the amino acid substitutions L234A and L235A, and HCVR and LCVR CDRs CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 13 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO: 14.

45 **[0090]** Disclosed but not part of the claimed invention is the humanized anti-SIRPα antibody for use in the treatment of human solid tumours and haematological malignancies in combination with the use of a therapeutic antibody against a membrane-bound target on the surface of tumour cells which comprises a human Fc region that binds to activating Fc receptors present on human immune effector cells, comprises an Fc region comprising the amino acid substitutions L234A and L235A, and HCVR and LCVR CDRs selected from the group consisting of:

50

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:3 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:4;
b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6;
55 c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:7 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:8;
d. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:9 and CDR1, and CDR2 and CDR3 amino acid sequences of SEQ ID NO: 10.

**[0091]** In a preferred embodiment, HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:36;

a. HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:37;
b. HCVR amino acid sequence of SEQ ID NO: 13 and LCVR amino acid sequence of SEQ ID NO:38; or
c. HCVR amino acid sequence of SEQ ID NO: 13 and LCVR amino acid sequence of SEQ ID NO:37.

**[0092]** Disclosed but not part of the claimed invention is the humanized anti-SIRP$\alpha$ antibody for use as defined hereinabove comprises an Fc region comprising the amino acid substitutions L234A and L235A, and

d. HCVR amino acid sequence of SEQ ID NO:30 and LCVR amino acid sequence of SEQ ID NO:31;
e. HCVR amino acid sequence of SEQ ID NO:32 and LCVR amino acid sequence of SEQ ID NO:33; or
f. HCVR amino acid sequence of SEQ ID NO:34 and LCVR amino acid sequence of SEQ ID NO:8;

**[0093]** More preferably, the modified Fc IgGi region does not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgGi region does not comprise an amino acid substitution at position N297.

**[0094]** In yet another preferred embodiment, the humanized anti-SIRP$\alpha$ antibody for use as defined hereinabove comprises an Fc region comprising the amino acid substitutions L234A and L235A, and HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:36.

**[0095]** In yet another preferred embodiment, the humanized anti-SIRP$\alpha$ antibody for use as defined hereinabove comprises an Fc region comprising the amino acid substitutions L234A and L235A, and HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:37.

**[0096]** In yet another preferred embodiment, the humanized anti-SIRP$\alpha$ antibody for use as defined hereinabove comprises an Fc region comprising the amino acid substitutions L234A and L235A, and HCVR amino acid sequence of SEQ ID NO: 13 and LCVR amino acid sequence of SEQ ID NO:38.

**[0097]** In yet another preferred embodiment, the humanized anti-SIRP$\alpha$ antibody for use as defined hereinabove comprises an Fc region comprising the amino acid substitutions L234A and L235A, and HCVR amino acid sequence of SEQ ID NO: 13 and LCVR amino acid sequence of SEQ ID NO:37.

**[0098]** More preferably, the humanized anti-SIRP$\alpha$ antibodies according to the invention as defined hereinabove for use as defined hereinabove comprising an Fc region comprising the amino acid substitutions L234A and L235A,the modified Fc IgGi region do not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgGi region does not comprise an amino acid substitution at position N297.

**[0099]** The anti-SIRP$\alpha$ antibodies comprising a modified Fc region that exhibits reduced binding to a human Fc$\alpha$ or Fcy receptor, when compared to the same anti-SIRP$\alpha$ antibody comprising a wild-type Fc region as described hereinabove enhance the *in vitro* ADCC of a therapeutic antibody using neutrophils as effector cells from different donors homozygous for either SIRP$\alpha_{BIT}$ or SIRP$\alpha_1$. All of these antibodies increase the *in vitro* ADCC using neutrophils of most donors, the preferred antibodies even increase *in vitro* ADCC using neutrophils of all donors.

**[0100]** Any references in the description to methods of treatment refer to the antibodies or pharmaceutical compositions of the present invention for use in a method of treatment of the human (or animal) body by therapy.

## EXAMPLES

### Immunization protocol and selection

**[0101]** Rabbits were repeatedly immunized with a mixture of peptides representing the extra cellular domain region of human (hu)SIRP$\alpha_{BIT}$, human (hu)SIRP$\alpha_1$ and cynomolgus (cy)SIRP$\alpha$. Blood was collected at different time points and enriched with lymphocytes. Single B-cells were deposited into single wells of microtiter plates. These B-cells were cultivated for several days in the presence of conditioned medium and feeder cells. During this time they produced and released monoclonal antibodies into the cultivation medium (B-cell supernatants). The supernatants of these single B-cells were analyzed for IgG production; subsequently the specific binding huSIRP$\alpha_{BIT}$ and huSIRP$\alpha_1$, to cySIRP$\alpha$ and to an anti-Fc antibody was determined. Suitable supernatants were those binding to both huSIRP$\alpha_{BIT}$ and huSIRP$\alpha_1$ and to cySIRP$\alpha$. After a hit picking step binding to mouse (mu) SIRP$\alpha$ and to huSIRP$\beta_{1v1}$, huSIRP$\beta_{1v2}$ and huSIRPy (as anti-targets) was measured. In addition, the binding to SIRP$\alpha_{BIT}$ and SIRP$\alpha_1$-over expressing CHO cells was determined. Binding to parental CHO cells was applied as a control assay.

**[0102]** Suitable B-cell lysates were selected for RNA isolation, reverse transcription and sequencing. The unique variable regions of antibody light and heavy chains were gene synthesized and cloned in front of the antibody constant region sequence (kappa LC SEQ ID NO:26 and human IgGi HC-LALA format SEQ ID NO:27), respectively.

**[0103]** HEK 293 cells were transiently transfected with the antibody sequence containing plasmids using an automated

procedure on a Tecan Freedom Evo platform. Immunoglobulins were purified from the cell supernatant using affinity purification (Protein A) on a Dionex Ultimate 3000 HPLC system with a plate autosampler. The produced antibodies were tested in ELISA-type assays (ELISA: huSIRP$\alpha_1$, huSIRP$\alpha_{BIT}$, cySIRP$\alpha$, muSIRP$\alpha$, huSIRP$\beta_{1v1}$/$\beta_{1v2}$/$\gamma$; cell binding assays: huSIRP$\alpha_1$, huSIRP$\alpha_{BIT}$).

**Transient expression of antibodies**

a) Preparation of cDNA constructs and expression vectors

**[0104]** The HCVR amino acid sequences of the antibodies were each joined_at the N-terminus to a leader sequence (SEQ ID NO:28 for antibodies 1-9, 15, 16; SEQ ID NO:39 for antibodies 10-14), and at the C-terminus to the constant domain of a human IgGi HC LALA according to SEQ ID NO:27. The HCVR amino acid sequences of antibodies 12C4huIgGiLALA, 12C4huIgGi or 29AM4-5huIgGiLALA were each joined at the N-terminus to a HAVT20 leader sequence (SEQ ID NO:29) and at the C-terminus to the constant domain of a human IgGi HC LALA according to SEQ ID NO:27 or a wild type human IgGi HC (SEQ ID NO:25). The resulting chimeric amino acid sequences were back-translated into a cDNA sequence codon-optimized for expression in human cells (*Homo sapiens*). Similarly, the chimeric cDNA sequence for the LC of the construct was obtained by joining the sequences of a leader sequence (SEQ ID NO:28 for antibodies 1-9, 12; SEQ ID NO:40 for antibodies 10, 11, 13-16, SEQ ID NO:29 for 12C4huIgGiLALA, 12C4huIgGi and 29AM4-5huIgGiLALA) to the LCVR of antibodies 1-16, 12C4huIgGiLALA and 12C4huIgGi and 29AM4-5huIgGiLALA at the N-terminus and at the C-terminus to a human IgGi $\kappa$ light chain constant region (SEQ ID NO:26). The HCVR and LCVR sequences according to Table 1 were used.

Table 1 HCVR and LCVR sequences of the antibodies and reference antibodies

| Antibody | HCVR | LCVR |
|---|---|---|
| 1 | SEQ ID NO:1 | SEQ ID NO:2 |
| 2 | SEQ ID NO:3 | SEQ ID NO:4 |
| 3 | SEQ ID NO:5 | SEQ ID NO:6 |
| 4 | SEQ ID NO:7 | SEQ ID NO:8 |
| 5 | SEQ ID NO:9 | SEQ ID NO:10 |
| 6 | SEQ ID NO:11 | SEQ ID NO:12 |
| 7 | SEQ ID NO:13 | SEQ ID NO:14 |
| 8 | SEQ ID NO:15 | SEQ ID NO:16 |
| 9 | SEQ ID NO:17 | SEQ ID NO:18 |
| 29AM4-5huIgGiLALA | SEQ ID NO:19 | SEQ ID NO:20 |
| 12C4huIgGiLALA | SEQ ID NO:21 | SEQ ID NO:22 |
| 12C4huIgGi | SEQ ID NO:21 | SEQ ID NO:22 |
| KWAR23 | SEQ ID NO:23 | SEQ ID NO:24 |
| 10 humanized | SEQ ID NO:30 | SEQ ID NO:31 |
| 11 humanized | SEQ ID NO:32 | SEQ ID NO:33 |
| 12 humanized | SEQ ID NO:34 | SEQ ID NO:8 |
| 13 humanized | SEQ ID NO:35 | SEQ ID NO:36 |
| 14 humanized | SEQ ID NO:35 | SEQ ID NO:37 |
| 15 humanized | SEQ ID NO:13 | SEQ ID NO:38 |
| 16 humanized | SEQ ID NO:13 | SEQ ID NO:37 |

b) Vector construction and cloning strategy

**[0105]** For expression of the antibody chains a mammalian expression vector was used, which contains a CMV:BGHpA

expression cassette. The final vectors containing either the HC or the LC expression cassette (CMV:HC:BGHpA and CMV:LC-BGHpA, respectively) were transferred to and expanded in *E. coli* NEB 5-alpha cells. Large-scale production of the final expression vectors for transfection was performed using Maxi- or Megaprep kits (Qiagen).

5 c) Transient expression in mammalian cells

**[0106]** Commercially available Expi293F cells (Thermo Fisher) were transfected with the expression vectors using the ExpiFectamine transfection agent according to the manufacturer's instructions as follows: $75\times10^7$ cells were seeded in 300 mL FortiCHO medium, 300 μg of the expression vector was combined with 800 μl of ExpiFectamine transfection
10 agent and added to the cells. One day after transfection, 1.5 ml Enhancer 1 and 15 ml Enhancer 2 were added to the culture. Six days post transfection, the cell culture supernatant was harvested by centrifugation at 4,000 g for 15 min and filtering the clarified harvest over PES bottle filters/ MF 75 filters (Nalgene).

**Antibody binding and specificity**

15

*Experimental*

**[0107]** ELISA assay: Solutions of huSIRP$\alpha_1$, huSIRP$\alpha_{BIT}$, huSIRP$\beta_{1v1}$, huSIRP$\beta_{1v2}$, huSIRPy and cySIRP$\alpha$ in phosphate buffered saline (PBS) were each added to a multiple well black polystyrene plate for ELISA and allowed to adhere
20 for 1 h at RT. Unbound protein was removed with three washing steps using standard washing buffer. Subsequently, blocking buffer was added to the wells. After 1 h incubation at RT, the wells were washed three times with standard washing buffer. The antibodies in buffer at various concentrations were added to the wells and incubated at RT for 1 h. Unbound antibodies were removed with three washing steps using standard washing buffer. Goat anti human IgG (Fab')$_2$:horse radish peroxidase (HRP) in buffer was added to the wells and incubated at RT for 1 h. 3,3',5,5'-Tetrame-
25 thylbenzidine (TMB) was added and after sufficient colour development HCl was added. Absorbance was read at 450 nm/620 nm.

**[0108]** Surface Plasmon Resonance (SPR) assay: Affinity analysis was performed by single cycle kinetics analysis on a Surface Plasmon Resonance apparatus (Biacore T200 system, GE Life Sciences) at 25°C. Biotinylated SIRP antigens were captured on the surface of a chip suitable for biotinylated molecules (Sensor Chip CAP, GE Life Sciences)
30 by injecting 5 μg/ml of the SIRP antigen in running buffer (10 mM HEPES buffer at pH 7.4 with 150 mM NaCl, 3 mM EDTA and 0.005% v/v polyoxyethylene (20) sorbitan monolaurate (Surfactant P20) for 60 sec at 10 μL/min after injection of a streptavidin conjugate (20x diluted biotin CAPture reagent, GE Life Sciences) for 60 sec at 10 μl/min. Baseline stabilization was set at 1 min after which five increasing concentrations of an anti-SIRP antibody in running buffer (10 mM HEPES buffer at pH 7.4 with 150 mM NaCl, 3 mM EDTA and 0.005% v/v polyoxyethylene (20) sorbitan monolaurate)
35 were injected. For each step an association time of 150 sec was used, followed by a dissociation time of 1200 sec at the highest concentration only, all at a flow rate of 30 μL/min. Regeneration was performed with 6 M guanidine-HCl, 0.25 M NaOH solution (60 sec with flow rate of 30 μL/min). Double blank subtraction was performed on the observed sensorgrams using a non anti-SIRP (blank) immobilized reference flow channel and running buffer injection. Sensorgrams were fitted with a 1:1 Langmuir model for all tested anti-SIRP antibodies. The kinetic parameters ($k_a$, $k_d$ and $K_D$) were
40 calculated using the Biacore T200 evaluation software (v3.1).

**[0109]** Flow Cytometry: U937 cells endogenously expressing human SIRP$\alpha_{BIT}$ antigen and cells derived from a non-engineered subclone that has been screened and isolated from CHO-S Chinese hamster ovary cells (ExpiCHO-S) cells expressing human SIRP$\alpha_1$, SIRP$\alpha_{BIT}$ or cySIRP$\alpha$ antigen (100,000 cells/well in a 96-well plate) were washed three times with ice-cold FACS buffer (1× PBS (LONZA) containing 0.2% v/w BSA (Sigma-Aldrich, St. Louis, MO) and 0.02%
45 v/w $NaN_3$ (Sigma-Aldrich), followed by the addition of a concentration range of each primary mAb (50 μL/well) diluted in ice-cold FACS buffer. After an incubation time of 30 min at 4°C, the cells were washed three times with ice-cold FACS buffer and 50 μL/well secondary mAb (AffmiPure F(ab')$_2$ fragment Goat-anti-human IgG-APC, 1:6,000 dilution, Jackson Immuno Research) was added. After 30 min at 4°C, cells were washed twice and resuspended in 150 μL FACS buffer. Fluorescence intensities were determined by flow cytometry (BD FACSVerse, Franklin Lakes, NJ) and indicated as the
50 median fluorescence intensity (MFI-Median) for U937 cells and ExpiCHO-S cells. Curves were fitted by nonlinear regression using the sigmoidal dose-response equation with variable slope (four parameters) in GraphPad Prism (version 7.02 for Windows, GraphPad, San Diego, CA). $EC_{50}$ values were calculated as the concentration in μg/mL that gives a response halfway between bottom and top of the curve, when using a 4-parameter logistic fit.

55 *Results*

**[0110]** ELISA assay: The $EC_{50}$ values for binding to huSIRP$\alpha_1$, huSIRP$\alpha_{BIT}$, huSIRP$\beta_1$, huSIRP$\beta_{1v2}$, huSIRPy, cySIRP$\alpha$ obtained with ELISA for antibodies 1-9 and reference antibodies are summarized in Table 2. All antibodies bind

to huSIRP$\alpha_1$ and to huSIRP$\alpha_{BIT}$. Antibodies 29AM4-5huIgGiLALA and 12C4huIgGiLALA, bind to huSIRP$\beta_{1v1}$, huSIRP$\beta_{1v2}$, and huSIRPy. The antibodies 2-6, 8 and 9 show a low affinity for huSIRP$_{\beta 1v1}$ and for huSIRPy. Antibody 7 binds to huSIRP$\beta_{1v1}$, but has low affinity for huSIRP$\beta_{1v2}$ and huSIRPy. Antibody 1 binds to huSIRP$\beta_{1v2}$ and huSIRPy.

Table 2 Specificity of the anti-SIRP$\alpha$ antibodies and reference antibodies

| Antibody | huSIRP$\alpha_1$ $EC_{50}$ (ng/ml) | huSIRP$\alpha_{BIT}$ $EC_{50}$ (ng/ml) | huSIRP$\beta_{1v1}$ $EC_{50}$ (ng/ml) | huSIRP$\beta_{1v2}$ $EC_{50}$ (ng/ml) | huSIRP$\gamma$ $EC_{50}$ (ng/ml) | cySIRP$\alpha$ $EC_{50}$ (ng/ml) |
|---|---|---|---|---|---|---|
| 1 | 39 | 21 | 100,000 | 58 | 43 | 305 |
| 2 | 33 | 27 | 100,000 | 28 | 100,000 | 38 |
| 3 | 15 | 24 | 100,000 | 89 | 5,216 | 36 |
| 4 | 53 | 25 | 100,000 | 92 | 100,000 | 99 |
| 5 | 31 | 21 | 3,518 | 110 | 100,000 | 123 |
| 6 | 21 | 20 | 100,000 | 24 | 100,000 | 33 |
| 7 | 23 | 20 | 14 | 100,000 | 100,000 | 335 |
| 8 | 19 | 20 | 100,000 | 19 | 100,000 | 26 |
| 9 | 23 | 26 | 100,000 | 47 | 100,000 | 30 |
| 29AM4-5* | 9 | 9 | 13 | 17 | 34 | 11 |
| 12C4* | 7 | 5 | 8 | 6 | 6 | 5 |

*huIgGiLALA
$EC_{50}$ values > 100,000 have been adjusted to 100,000.

**[0111]** SPR assay: The $K_D$ values for binding to huSIRP$\alpha_1$, huSIRP$\alpha_{BIT}$ and huSIRPy of antibodies 4, 7, 10-14 in comparison with reference antibodies KWAR23, huIgGi12C4LALA and SE5A5 (purchased from a commercial supplier) are summarized in Table 3. Antibodies 4, 7, 10-14 bind to both huSIRP$\alpha_1$ and huSIRP$\alpha_{BIT}$, and do not bind to huSIRPy. All reference antibodies do bind to huSIRPy.

Table 3 SPR data ($K_D$ in M)

| Antibody | $K_D$ (huSIR$\alpha_{BIT}$) | $K_D$ (huSIRP$\alpha_1$) | $K_D$ (huSIRP$\gamma$) |
|---|---|---|---|
| KWAR23 mouse IgG2a | <1.0E-11[1] | <1.0E-11 | <1.0E-11 |
| KWAR23 huIgGiLALA | <1.0E-11[1] | 1.1E-11 | <1.0E-11 |
| 12C4huIgGiLALA | 1.5E-11 | 8.7E-11 | 1.6E-11 |
| SE5A5 | 2.6E-9 | 2.2E-9 | 4.9E-8 |
| 4 | <1.0E-11 | 2.6E-11 | N[2] |
| 7 | <1.0E-11 | <1.0E-11 | N |
| 10 humanized | <1.0E-11 | 3.2E-9 | N |
| 11 humanized | 1.4E-10 | 4.1E-8 | N |
| 12 humanized | <1.0E-11 | 5.9E-11 | N |
| 13 humanized | 1.2E-11 | <1.0E-11 | N |
| 14 humanized | 8.9E-11 | <1.0E-11 | N |

[1] <1.0E-11: $K_D$ is outside the range which means high affinity
[2] N: No specific binding found

**[0112]** Flow Cytometry assay: The binding of various antibodies to huSIRP$\alpha_1$, huSIRP$\alpha_{BIT}$, and/or cySIRP$\alpha$ expressed

on cells was determined by flow cytometry. The binding is indicated in $EC_{50}$ values, which are shown in Table 4. Antibodies 2, 4, 5, 7, 8, 10-14 bind to huSIRP$\alpha_1$, huSIRP$\alpha_{BIT}$ and cySIRP$\alpha$. Antibodies 2, 4, 5, 7, 8, 10-14 bind to cySIRP$\alpha$ in the low $\mu$g/mL range.

5

Table 4 Flow Cytometry data

| Antibody | U937 cells (SIRP$\alpha_{BIT}$) $EC_{50}$ ($\mu$g/mL) | ExpiCHO-S (huSIRP$\alpha_1$) $EC_{50}$ ($\mu$g/mL) | ExpiCHO-S (huSIR$\alpha_{BIT}$) $EC_{50}$ ($\mu$g/mL) | ExpiCHO-S (cySIRP$\alpha$) $EC_{50}$ ($\mu$g/mL) |
|---|---|---|---|---|
| 1 | - | - | - | - |
| 2 | 0.14 | 0.19 | 0.27 | 0.16 |
| 3 | 0.22 | - | - | - |
| 4 | 0.12 | 0.41 | 0.23 | 0.18 |
| 5 | 0.16 | 0.27 | 0.22 | 0.26 |
| 6 | - | - | - | - |
| 7 | 0.17 | 0.23 | 0.21 | 0.07 |
| 8 | 0.12 | 0.22 | 0.18 | 0.15 |
| 9 | 0.11 | - | - | - |
| 29AM4-5 huIgG1LALA | 0.25 | - | - | - |
| 12C4huIgG1 LALA | 0.19 | - | - | - |
| KWAR23 huIgG1LALA | 0.09 | - | - | - |
| 10 | 0.17 | 0.38 | 0.2 | 0.27 |
| 11 | 0.13 | 1.05 | 0.3 | 0.32 |
| 12 | 0.2 | 0.1 | 0.46 | 0.17 |
| 13 | 0.14 | 0.36 | 0.23 | 0.44 |
| 14 | 0.22 | 0.37 | 0.29 | 0.38 |
| 15 | 0.16 | - | - | - |
| 16 | 0.23 | - | - | - |
| - value not determined | | | | |

10 15 20 25 30 35 40

**Antibody blocking of CD47-SIRP$\alpha$ binding**

45

*Experimental*

**[0113]** CHO cells transfected with either SIRP$\alpha_1$ or SIRP$\alpha_{BIT}$ or parental CHO cells as control were seeded in 20 $\mu$l cell medium in a well plate with clear bottom and incubated overnight. Antibodies 1-9, 29AM4-5huIgGiLALA or 12C4huIgGiLALA reference antibodies together with a mixture of His tag® CD47 and anti-His tag® fluorescent detection antibody were added to the wells and incubated for 2 h. After incubation, the cells were washed with cell wash buffer. Fluorescence was determined using a screening system (CellInsight®, Thermo Scientific®) and total fluorescence per cell was determined.

50

*Results*

55

**[0114]** Antibodies 29AM4-5huIgGiLALA, 12C4huIgGiLALA, 3 and 7 block binding of CD47 to both CHO cells expressing huSIRP$\alpha_1$ and CHO cells expressing huSIRP$\alpha_{BIT}$ completely, antibodies 1, 2, 4-6, 8 and 9 do neither block binding of CD47 to CHO cells expressing huSIRP$\alpha_1$ nor to CHO cells expressing huSIRP$\alpha_{BIT}$.

**ADCC assay**

**[0115]** Neutrophils of donors homozygous for either SIRP$\alpha_1$ or SIRP$\alpha_{BIT}$ were isolated and cultured according to the method in Chao et al. PNAS 2011, 108(45), 18342-18347. ADCC was determined using the $^{51}$Cr release assay or the non-radioactive Europium TDA (EuTDA) cytotoxicity assay (DELFIA, PerkinElmer). SKBR3 cells were used as target cells and labelled with 100 $\mu$Ci $^{51}$Cr (Perkin-Elmer) for 90 min at 37°C, or with bis (acetoxymethyl) 2,2':6',2"- terpyridine-6,6"-dicarboxylate) (BATDA reagent Delfia), for 5 min at 37°C. After 2 washes with PBS, $5\times10^3$ target cells per well were incubated in IMDM culture medium supplemented with 10% (v/v) foetal calf serum (FCS) for 4 hours at 37°C and 5% $CO_2$ in a 96-well U-bottom plate together with neutrophils in an effector to target cell ratio of 50:1 in the presence of the appropriate antibodies. After the incubation, supernatant was harvested and analyzed for radioactivity in a gamma counter (Wallac) or was added to europium solution (DELFIA, PerkinElmer) and the europium 2,2':6',2"-terpyridine-6,6"-dicarboxylic acid (EuTDA) fluorescence was determined using a spectrofluorometer (Envision, PerkinElmer). The percentage of cytotoxicity was calculated as [(experimental release - spontaneous release) / (total release - spontaneous release)] $\times$ 100%. All conditions were measured in duplicate and/or triplicate.

*ADCC data 12C4huIgG$_1$LALA versus 12C4IgG$_1$*

**[0116]** Figure 1 shows the results of the ADCC assay as cytotoxicity in %. The % cytotoxicity measured on SKBR3 cells using neutrophils as effector cells and trastuzumab alone is less than the % cytotoxicity of trastuzumab in combination with the murine 12C4 antibody (mu12C4). Trastuzumab in combination with an antibody wherein 12C4 variable regions are grafted onto a human IgGi constant region (12C4huIgGi) shows similar % cytotoxicity as compared to trastuzumab alone at low concentrations of 12C4huIgGi. At higher concentrations 12C4huIgGi, a decrease in % cytotoxicity is observed. Trastuzumab in combination with an antibody wherein 12C4 variable regions are grafted onto a human IgGi constant region comprising amino acid substitutions L234A and L235A (12C4huIgGiLALA) shows increased % cytotoxicity compared to the % cytotoxicity of trastuzumab alone, and increased % cytotoxicity compared to the combination of 12C4huIgGi and trastuzumab.

*ADCC data*

**[0117]** Figure 2 compares the % ADCC by human neutrophils relative to trastuzumab (set to 100%) in the presence of antibody 1-9 having a human IgGi constant region comprising amino acid substitutions L234A and L235A (LALA) in combination with trastuzumab in comparison with 12C4huIgGiLALA. B6H12IgGiLALA, having the VR of a murine anti-CD47 antibody and a human IgGi constant region comprising amino acid substitutions L234A and L235A, and vehicle (no trastuzumab) were used as positive and negative control, respectively. Filled squares, (■), are the values measured with neutrophils of donors having the SIRP$\alpha_{BIT}$ variant (homozygous for SIRP$\alpha_{BIT}$), open circles (○) are the values measured with neutrophils of donors having the SIRP$\alpha_1$ variant (homozygous for SIRP$\alpha_1$). For all antibodies the average ADCC was increased in comparison to trastuzumab alone. For antibodies 1, 2, 4, 5, 7 and 8 the average ADCC increase was enhanced even more than the 12C4huIgGiLALA-induced ADCC increase. When the ADCC increase per donor per antibody is compared, antibodies 1, 3-6, 8 and 9 show less variation in % increase in ADCC than 12C4huIgGiLALA.

**[0118]** Figure 3 compares the % ADCC by human neutrophils in the presence of various concentrations of chimeric antibodies 4 and 7 and humanized antibodies 10 and 14 having a human IgGi constant region comprising amino acid substitutions L234A and L235A (LALA) in combination with trastuzumab in comparison with trastuzumab alone and trastuzumab in combination with various concentrations of 12C4huIgGiLALA. Neutrophils of two donors homozygous for SIRP$\alpha_{BIT}$ were used. Even at low concentrations antibodies 4, 7, 10 and 14 increase ADCC. The ADCC increase is concentration dependent.

**[0119]** Figure 4 compares the % ADCC by human neutrophils in the presence of antibodies 4, 7, 10, 13, 14, 15 and 16 in combination with trastuzumab (Tmab) in comparison with the % ADCC trastuzumab alone and 12C4huIgGiLALA. All antibodies increase the ADCC in comparison with trastuzumab alone. The ADCC increase by neutrophils of most donors in the presence of antibodies 4, 7, 10, 13, 14, 15 and 16 in combination with trastuzumab is similar or increased in comparison with 12C4huIgGiLALA in combination with trastuzumab.

Sequence listings with underlined CDR1, CDR2 and CDR3 amino acid sequences in heavy chain (HC) and light chain (LC) variable region (VR) amino acid sequences (determined using the method of Kabat)

**[0120]**

SEQ ID NO:1 (HC VR 1)

1 QSVEESGGRL VTPGTPLTLT CTVSGIDLSS YAMSWVRQAP GKGLEWIGII

51 SSGGITYYAS WAKGRFTISK TSTTVDLKIP SPTTEDTATY FCARSLWAAS

101 NYYMALWGPG TLVTVSS

SEQ ID NO:2 (LC VR 1)

1 AIKMTQTPAS VSAAVGGTVS INCQASEDIE SYLAWYQQKP GQPPKLLIYR

51 ASTLASGVSS RFKGSGSGTQ FTLTISDLES ADAATYYCLG DYYSSSGDTG

101 AFGGGTEVVV K

SEQ ID NO:3 (HC VR 2)

1 QSVEESGGRL VTPGTPLTLT CTVSGFSLSN YAMHWVRQAP GKGLEWIGII

51 YTGGATSYAT WAKGQFTISK TSTTVDLKIT SPTTEDTATY FCARGDRDGY

101 AYFNIWGPGT LVTVSL

SEQ ID NO:4 (LC VR 2)

1 QIVMTQTPFS VSAVVGGTVT IKCQASHNIG SWLAWYQQKP GQRPKLLIYD

51 ASTLASGVSS RFKGSGSGTE FTLTISGVES ADAATYYCQQ GYGISYVHNV

101 FGGGTEVVVK

SEQ ID NO:5 (HC VR3)

1 QSVEESGGRL VTPGTPLTLA CTVSGFSLIS YYISWVRQAP EKGLEYIGII

51 NIGGGASYAS WAKGRFTISK TSTTVDLKIT SPTPEDTATY FCAMSYGMDT

101 GAFNIWGPGT LVTVSL

SEQ ID NO:6 (LC VR 3)

1 AQVLTQTPAS VSAAVGGTVT ISCQSSESVY KNNFLSWYQQ KPGKPPKLLI

51 YGASTLASGV PSRFKGSGSG TQFTLTISDL ESDDAATYFC QGGYRTDIYP

101 FGGGTEVVVK

SEQ ID NO:7 (HC VR 4)

1 QSVEESGGRL GTPGTPLTLT CTVSGFSLSS YVMGWFRQAP GKGLEYIGII
51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SPTTEDTATY FCARVGPLGV
101 DYFNIWGPGT LVTVSL

SEQ ID NO:8 (LC VR 4)

1 DIVMTQTPSS VEAAVGGTVT IKCQAGQSIN SYLAWYQQKP GQRPKLLIYY
51 ASTLESGVPS RFKGSGSGTD YTLTISDLES ADAATYYCQS WHYISRSYAF
101 GGGTEVVVK

SEQ ID NO:9 (HC VR 5)

1 QSVEESGGRL VTPGTPLTLT CTVSGFSLSS YVMGWFRQAA GKGLEYIGYI
51 NADGSPYYAT WVNGRFTISK TPTTMDLKIN SPTTEDTATY FCARVGPLGV
101 DYFNIWGPGT LVTVSL

SEQ ID NO:10 (LC VR 5)

1 DIVMTQTPAS VEAAVGGTVT IKCQASQSIN RYLTWYQQKP GQRPKLLIYY
51 ASTLESGVPS RFEGSGSGTD YTLTISDLES ADAATYYCQS YYYISRTYAF
101 GGGTEV VVK

SEQ ID NO:11 (HC VR 6)

1 QSVEESGGRL VTPGTPLTLT CTVSGIDLSS YTMTWVRQAP GKGLEWIGII
51 YAGGSTAYAS WAKGRFTISK TSTTVDLKIT SPTTEDTATY FCARSSSDGY
101 DYFNIWGPGT LVTVS L

SEQ ID NO:12 (LC VR 6)

1 GVVMTQTPSS VSAAVGGTVT INCQASQSIG SWLAWYQQKP GQPPKLLIYQ
51 ASKLASGVPS RFSGRGSGTH FTLTISDVQS DDAATYYCQQ TVTAASNVDNA
101 FGGGTEVVVK

SEQ ID NO:13 (HC VR 7)

1 RSVEESGGRL VTPGTPLTLT CTVSGFSLSS HGISWVRQAP GKGLEYIGTI

51 GTGVITYFAS WAKGRFTGSK TSTTVDLKIT SPTTEDTATY FCARGSAWND

101 PFDPWGPGTL VTVSS

SEQ ID NO:14 (LC VR 7)

1 ALVMTQTPAS VSAAVGGTVT TKCQASQSVY GNNDLAWYQH KPGQPPKLLI

51 YLASTLATGV PSRFSGSGSG TQFTLTITGV QSDDAATYYC LGGGDDEADN

101 VFGGGTEVVV K

SEQ ID NO:15 (HC VR 8)

1 QSLEESGGRL VTPGTPLTLT CTASGVDLSN YAMGWVRQAP GKGLEWIGII

51 YAGGSTSYAT WAKGRFTISK TSTTMDLKMT SPTTEDTATY FCARHRSDGY

101 DYFHLWGPGT LVTVSL

SEQ ID NO:16 (LC VR 8)

1 AIDMTQTPAS VSEPVGGTVT IKCQASQSIS SWLAWYQQKP GQRPKLLIYD

51 ASKLASGVPS RFSGSGSGTE FTLTISGVQS DDAAAYYCQQ GYAVSYVENI

101 FGGGTEVVVK

SEQ ID NO:17 (HC VR 9)

1 QSMEESGGRL VTPGTPLTLT CTASGFSLSN YGVSWVRQAP GKGLEWIGII

51 YGGSDITAYA SWAKGRFTIS KTSTTVDLTI TSPTTEDTAT YFCAKSYTNG

101 MDYYNIWGPG TLVTVSL

SEQ ID NO:18 (LC VR 9)

1 AFDLTQTPSS VEAPVGGTVI IKCQASQSIS SYLAWYQQKP GQPPKLLIYS

51 ASTLASGVSS RFKGSGSETQ FPLTISDLES ADAATYYCQS YYGSRSNVFG

101 GGTEVVVK

SEQ ID NO:19 (HC VR 29AM4-5)

1 EVQLVESGGG LVQPGGSLRL SCAASGFNIS YYFIHWVRQA PGKGLEWVAS

51 VYSSFGYTYY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARFT

101 FPGLFDGFFG AYLGSLDYWG QGTLVTVSS

SEQ ID NO:20 (LC VR 29AM4-5)

1 DIQMTQSPSS LSASVGDRVT ITCRASQSVS SAVAWYQQKP GKAPKLLIYS

51 ASSLYSGVPS RFSGSRSGTD FTLTISSLQP EDFATYYCQQ AVNWVGALVT

101 FGQGTKVEIK

SEQ ID NO:21 (HC VR 12C4)

1 EVKLEESGGG LMQPGGSMKL SCVASGFTFS NYWMNWVRQS PEKGLEWVAE

51 IRLKSNNYAT HYAESVKGRF TISRDDSKSS VYLQMNNLRA EDTGIYYCIR

101 DYDYDAYFDY WGQGTTLTVS S

SEQ ID NO:22 (LC VR 12C4)

1 DIVLTQSPAS LAVSLGQRAT ISCRASKSVS TSGYNYMYWY QQKPGQPPKL

51 LIYLASNLES GVPARFSGSG SGTDFTLNIH PVEEEDAATY YCQHSGELPY

101 TFGGGTKLEI K

SEQ ID NO:23 (HC VR KWAR23 )

1 EVQLQQSGAE LVKPGASVKL SCTASGFNIK DYYIHWVQQR TEQGLEWIGR

51 IDPEDGETKY APKFQDKATI TADTSSNTAY LHLSSLTSED TAVYYCARWG

101 AYWGQGTLVT VSS

SEQ ID NO:24 (LC VR KWAR23)

1 QIVLTQSPAI MSASPGEKVT LTCSASSSVS SSYLYWYQQK PGSSPKLWIY

51 STSNLASGVP ARFSGSGSGT SYSLTISSME AEDAASYFCH QWSSYPRTFG

101 AGTKLELK

SEQ ID NO:25 (human IgGi antibody HC constant region)

1 ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
51 HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP
101 KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
151 HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
201 EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC
251 LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
301 QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

SEQ ID NO:26 (human IgGi antibody LC κ constant region)

1 RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
51 NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK
101 SFNRGEC

SEQ ID NO:27 (human IgGi antibody HC constant region LALA mutant (mutations underlined)

1 ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
51 HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP
101 KSCDKTHTCP PCPAPEAAGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
151 HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
201 EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC
251 LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
301 QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

SEQ ID NO:28 (leader sequence HC 1-9, 15 + 16, LC 1-9 + 12)
1 MGWSCIILFL VATATGVHS

SEQ ID NO:29 (HAVT20 leader sequence)
1 MACPGFLWAL VISTCLEFSMA

SEQ ID NO:30 (HC VR 10)

1 KVEESGGGLV QPGGSLRLSC AASGFSLSSY VMGWVRQAPG KGLEWVSIIS
51 SSGSPYYASW VNGRFTISKD NSEGMVYLQM NSLRAEDTAV YYCARVGPLG
101 VDYFNIWGQG TTVTVSS

SEQ ID NO:31 (LC VR 10)

1 DIVMTQSPDS LAVSLGERAT INCQAGQSIN SYLAWYQQKP GQPPKLLIYY

51 ASTLESGVPD RFSGSGSGTD FTLTISSLQA EDVAVYYCQS WHYISRSYAF

101 GGGTKLEIK

SEQ ID NO:32 (HC VR 11)

1 EVKVEESGGG LVQPGGSLRL SCAASGFSLS SYVMGWVRQA PGKGLEWVSI

51 ISSSGSPYYA SWVNGRFTIS KTSTTMDLQM NSLRAEDTAV YYCARVGPLG

101 VDYFNIWGQG TTVTVSS

SEQ ID NO:33 (LC VR 11)

1 DIQMTQSPSS LSASVGDRVT ITCQAGQSIN SYLAWYQQKP GKVPKLLIYY

51 ASTLESGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCQS WHYISRSYAF

101 GQGTKVEIK

SEQ ID NO:34 (HC VR 12)

1 VQLVESGGRL VQPGTPLTLS CTVSGFSLSS YVMGWFRQAP GKGLEYIGII

51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SLRSEDTATY FCARVGPLGV

101 DYFNIWGPGT LVTVSS

SEQ ID NO:35 (HC VR 13 + 14)

1 RQLVESGGGL VQPGGSLRLS CTASGFSLSS HGISWVRQAP GKGLEYIGTI

51 GTGVITYFAS WAKGRFTGSK TSSTAYMELS SLRSEDTAVY FCARGSAWND

101 PFDPWGQGTL VTVSS

SEQ ID NO:36 (LC VR 13)

1 AIQMTQSPSS LSASVGDRVT ITCQASQSVY GNNDLAWYQQ KPGKAPKLLI

51 YLASTLATGV PSRFSGSGSG TDFTLTISSL QPEDFATYYC LGGGDDEADN

101 VFGGGTKVEI K

SEQ ID NO:37 (LC VR 14 + 16)

1 DIEMTQSPSS VSASVGDRVT LTCQASQSVY GNNDLAWYQQ KPGQAPKLLI

51 YLASTLATGV PSRFSGSGSG TDFTLTISSL QPEDFATYYC LGGGDDEADN

5

101 VFGGGTKVEI K

SEQ ID NO:38 (LC VR 15)

10

1 ELVMTQSPSS LSASVGDRVT ITCQASQSVY GNNDLAWYQQ KPGEAPKLLI

51 YLASTLATGV PSRFSGSGSG TDFTLTISGL QSEDFATYYC LGGGDDEADN

15

101 VFGQGTKVEI K

SEQ ID NO:39 (leader sequence heavy chains 10-14)
1 MGWTLVFLFL LSVTAGVHS

20

SEQ ID NO:40 (leader sequence light chains 10, 11, 13-16)
1 MVSSAQFLGL LLLCFQGTRC

**PATENTKRAV**

**1.** Anti-SIRPα-antistof eller et antigenbindende fragment deraf, som omfatter komplementaritetsbestemmende områder (CDR) CDR1, CDR2 og CDR3 af det variable område (VR) af tungkæden (HC) og letkæden (LC), hvor:
a. VR-CDR1 af HC består af aminosyresekvensen HGIS,
b. VR-CDR2 af HC består af aminosyresekvensen TIGTGVITYFASWAKG,
c. VR-CDR3 af HC består af aminosyresekvensen GSAWNDPFDP,
d. VR-CDR1 af LC består af aminosyresekvensen QASQSVYGNNDLA,
e. VR-CDR2 af LC består af aminosyresekvensen LASTLAT, og
f. VR-CDR3 af LC består af aminosyresekvensen LGGGDDEADNV,
hvor CDR'erne bestemmes ifølge Kabat-nummerering.

**2.** Anti-SIRPα-antistof eller antigenbindende fragment deraf ifølge krav 1, der er kimærisk, humaniseret eller humant.

**3.** Anti-SIRPα-antistof eller antigenbindende fragment deraf ifølge krav 2, der er humaniseret.

**4.** Humaniseret anti-SIRPα-antistof eller antigenbindende fragment deraf ifølge krav 3, og som omfatter
a. VR-aminosyresekvens af HC ifølge SEQ ID NO: 35 og VR-aminosyresekvens af LC ifølge SEQ ID NO: 36;
b. VR-aminosyresekvens af HC ifølge SEQ ID NO: 35 og VR-aminosyresekvens af LC ifølge SEQ ID NO: 37;
c. VR-aminosyresekvens af HC ifølge SEQ ID NO: 13 og VR-aminosyresekvens af LC ifølge SEQ ID NO: 38 eller
d. VR-aminosyresekvens af HC ifølge SEQ ID NO: 13 og VR-aminosyresekvens af LC ifølge SEQ ID NO: 37.

**5.** Anti-SIRPα-antistof ifølge et hvilket som helst af krav 1 til 4, og som omfatter et modificeret Fc-område, der har reduceret binding til en human Fcα- eller Fcγ-receptor sammenlignet med det samme anti-SIRPα-antistof omfattende et vildtype Fc-område.

**6.** Anti-SIRPα-antistof ifølge krav 5, og som omfatter et modificeret humant $IgG_1$-Fc-område omfattende aminosyresubstitutioner ved én eller flere positioner valgt fra gruppen bestående af L234, L235, G237, D265, D270, N297, A327, P328 og P329 ifølge Eu-nummerering.

**7.** Anti-SIRPα-antistof ifølge krav 6, og som omfatter aminosyresubstitutionerne L234A og L235A; L234E og L235A; L234A, L235A og P329A; eller L234A, L235A og P329G.

5 **8.** Anti-SIRPα-antistof ifølge krav 7, og som omfatter aminosyresubstitutionerne L234A og L235A; eller L234E og L235A.

**9.** Farmaceutisk sammensætning, der omfatter anti-SIRPα-antistoffet ifølge et hvilket som helst af krav 1 til 8 og et eller flere farmaceutisk acceptable hjælpestoffer.

10

**10.** Anti-SIRPα-antistof ifølge et hvilket som helst af krav 1 til 8 eller farmaceutisk sammensætning ifølge krav 9 til anvendelse som et lægemiddel.

**11.** Anti-SIRPα-antistof ifølge et hvilket som helst af krav 1 til 8 eller farmaceutisk
15 sammensætning ifølge krav 9 til anvendelse i behandling af humane solide tumorer eller hæmatologiske maligniteter.

**12.** Kombination af anti-SIRPα-antistoffet ifølge et hvilket som helst af krav 1 til 8 eller den farmaceutiske sammensætning ifølge krav 9 med ét eller flere andre lægemidler mod
20 cancer til anvendelse i behandling af humane solide tumorer eller hæmatologiske maligniteter.

**13.** Kombination til anvendelse ifølge krav 12, hvor det ene eller flere andre lægemidler mod cancer er målrettede lægemidler eller immunterapeutiske midler.

25

**14.** Kombination til anvendelse ifølge krav 13, hvor det målrettede lægemiddel er et terapeutisk antistof eller et antistof-lægemiddelkonjugat.

**15.** Kombination til anvendelse ifølge krav 14, hvor det terapeutiske antistof er et terapeutisk
30 antistof mod et membranbundet mål på overfladen af tumorceller, som omfatter et humant Fc-område, der binder til aktiverende Fc-receptorer til stede på humane immune effektorceller.

FIGURE 1

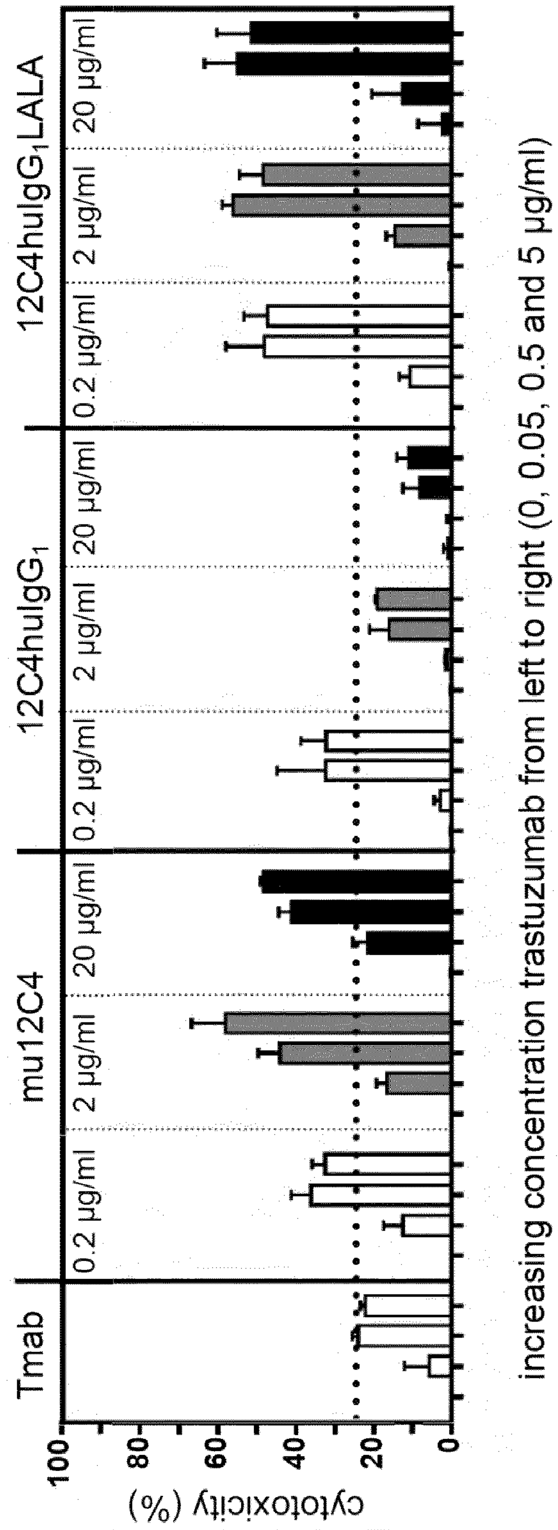


FIGURE 2

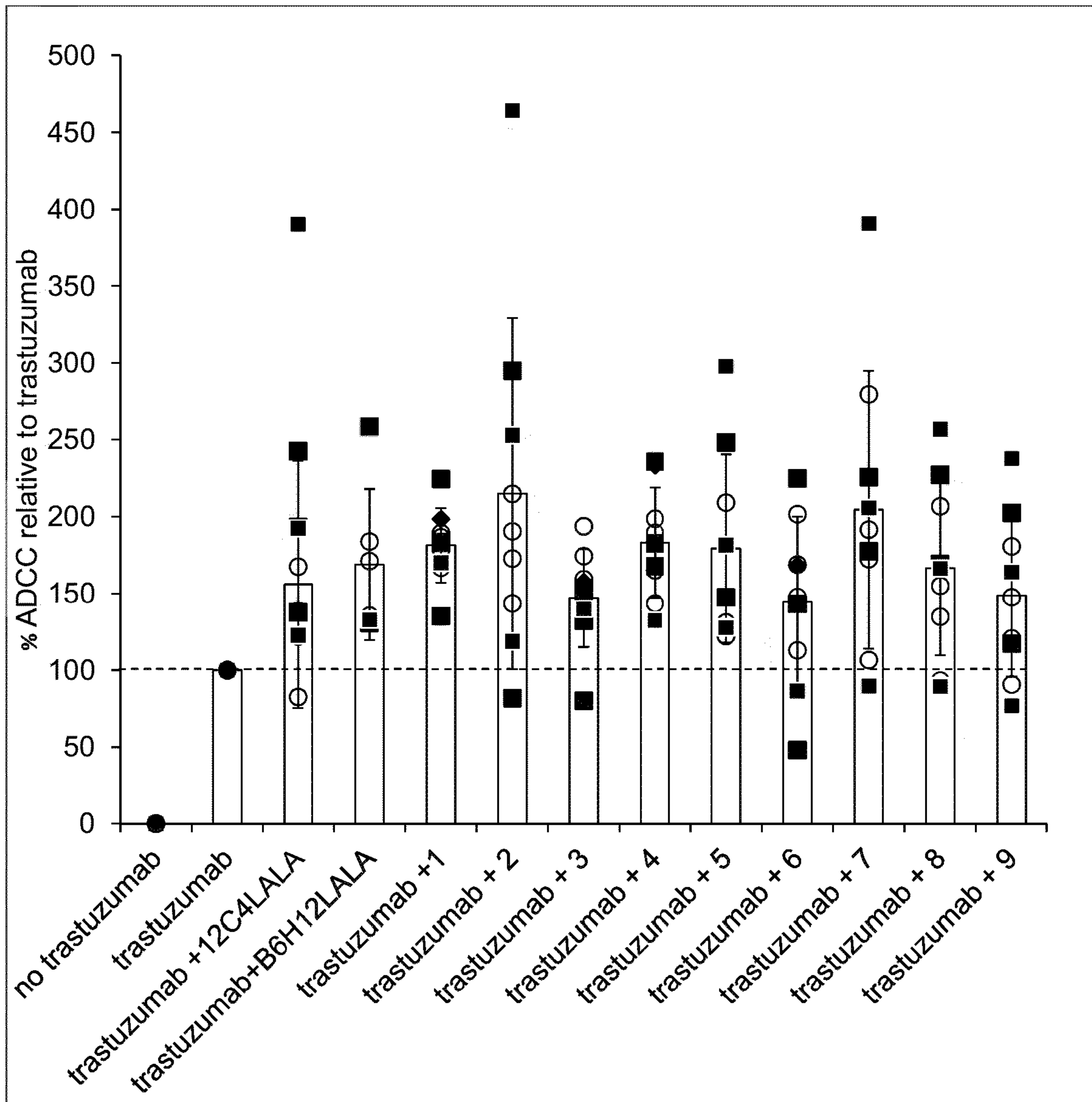


FIGURE 3

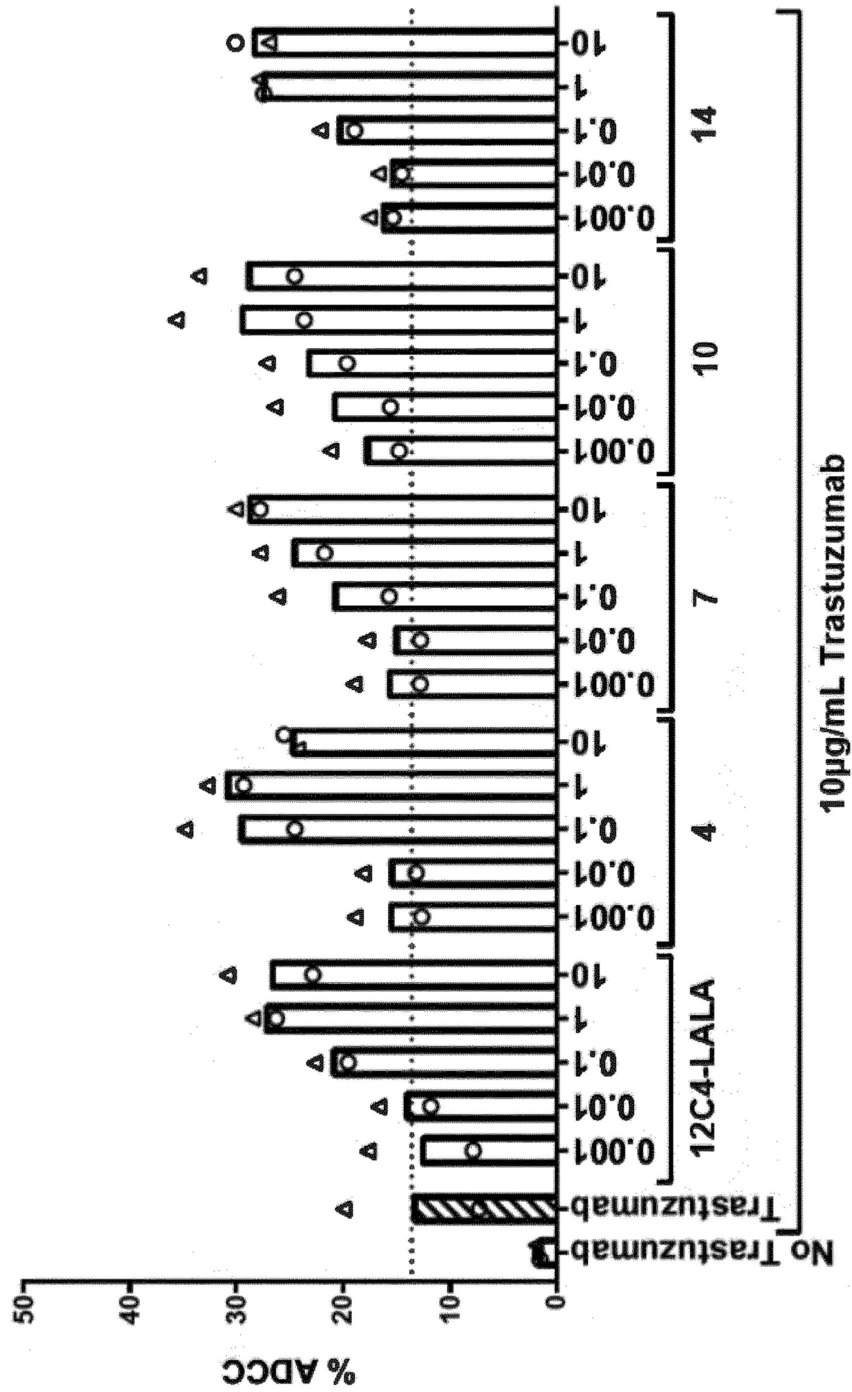
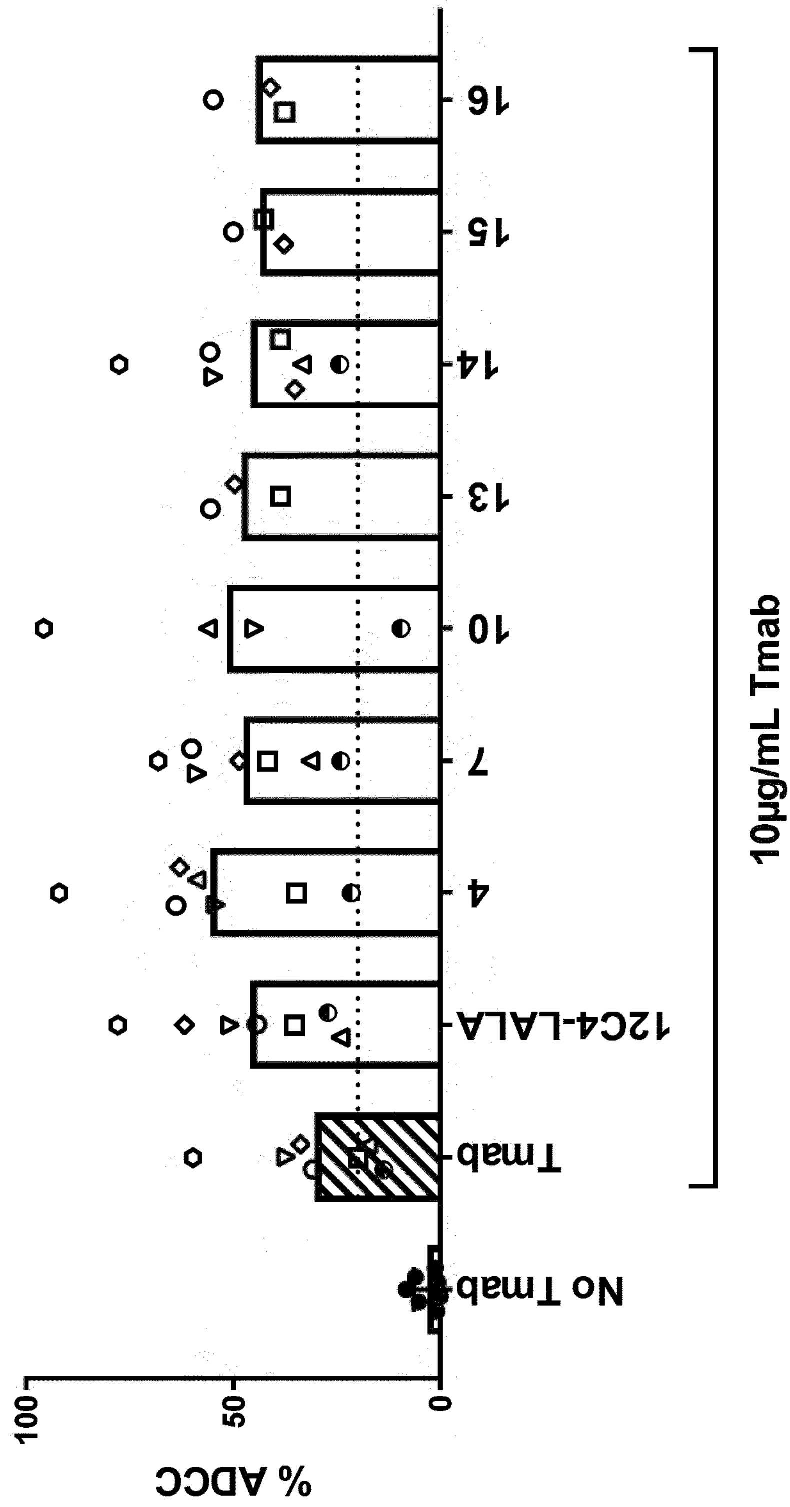


FIGURE 4



## REFERENCES CITED IN THE DESCRIPTION

*This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.*

### Patent documents cited in the description


- WO 2009131453 A **[0003]**
- WO 2015138600 A **[0006]**
- WO 2018026600 A **[0006]**
- WO 2013056352 A **[0006]**
- US 4816567 A **[0018]**


### Non-patent literature cited in the description


- **RICHARDS et al.** *Mol. Cancer Ther.,* 2008, vol. 7 (8), 2517-2527 **[0002]**
- **HAYES et al.** *J. Inflamm. Res.,* 2016, vol. 9, 209-219 **[0002]**
- **WEISKOPF.** *Eur. J. Cancer,* 2017, vol. 76, 100-109 **[0005]**
- **ZHAO et al.** *PNAS,* 2011, vol. 108 (45), 18342-18347 **[0006]**
- **TAKENAKA et al.** *Nature Immun.,* 2007, vol. 8 (12), 1313-1323 **[0007]**
- **HATHERLEY et al.** *J. Biol. Chem.,* 2014, vol. 289 (14), 10024-10028 **[0007]**
- **VAN BEEK et al.** *J. Immunol.,* 2005, vol. 175 (12), 7781-7787, 7788-7789 **[0008]**
- **MATOZAKI et al.** *J. Biol. Chem.,* 2004, vol. 279 (28), 29450-29460 **[0008]**
- **PICCIO et al.** *Blood,* 2005, vol. 105, 2421-2427 **[0009]**
- **STEFANISAKIS et al.** *Blood,* 2008, vol. 112, 1280-1289 **[0009]**
- **X.W. ZHAO et al.** *PNAS,* 2011, vol. 108 (45), 18342-18347 **[0010]**
- **YANAGITA et al.** *JCI Insight,* 2017, vol. 2 (1), e89140 **[0011]**
- **KÖHLER et al.** *Nature,* 1975, vol. 256, 495-497 **[0018]**
- **CLACKSON et al.** *Nature,* 1919, vol. 352, 624-628 **[0018]**
- **MARKS et al.** *J. Mol. Biol.,* 1991, vol. 222, 581-597 **[0018]**
- **KABAT, E.A. et al.** Sequences of Proteins of Immunological Interest. Public Health Service, National Institutes of Health, 1991 **[0021]**
- **CHOTHIA et al.** *Nature,* 1989, vol. 342, 877-883 **[0021]**
- **LEFRANC.** *The Immunologist,* 1999, vol. 7, 132-136 **[0021]**
- **SOTO-PANTOJA et al.** *Crit. Rev. Biochem. Mol. Biol.,* 2015, vol. 50 (3), 212-230 **[0023]**
- **E.S. DAY et al.** *Anal. Biochem.,* 2013, vol. 440, 96-107 **[0024]**
- **HARRISON et al.** *J. Pharm. Biomed. Anal.,* 2012, vol. 63, 23-28 **[0048]**
- **VAFA et al.** *Methods,* 2014, vol. 65, 114-126 **[0049]**
- **LEOH et al.** *Mol. Immunol.,* 2015, vol. 67, 407-415 **[0049]**
- **PAREKH et al.** *mAbs,* 2012, vol. 4 (3), 310-318 **[0049]**
- **PLEASS et al.** *J. Biol. Chem.,* 1999, vol. 271 (33), 23508-23514 **[0049]**
- **CHAO et al.** *PNAS,* 2011, vol. 108 (45), 18342-18347 **[0115]**