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(54) Title: ANTI-SIRP $\alpha$  ANTIBODIES

(57) Abstract: The present invention relates to antibodies against SIRP $\alpha$  that are suitable for use in anti-cancer therapy. The invention further relates to the use of the anti-SIRP $\alpha$  antibodies in the treatment of human solid tumours and haematological malignancies, optionally in combination with other anti-cancer therapeutics.

## ANTI-SIRP $\alpha$ ANTIBODIES

### FIELD OF THE INVENTION

5 The present invention relates to antibodies against SIRP $\alpha$  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 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 15 cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). However, therapeutic antibodies are often not effective 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 Fc $\gamma$  receptors, e.g. by amino acid substitutions (Richards *et al.* *Mol. Cancer Ther.* 2008, 7(8), 2517-2527) or by influencing the 20 glycosylation of the Fc region (Hayes *et al.* *J. Inflamm. Res.* 2016, 9, 209–219).

Another way of improving the ADCC and/or ADCP of a therapeutic antibody is by combining the therapeutic antibody with an antagonistic antibody against signal regulatory protein  $\alpha$  (anti-SIRP $\alpha$ ) or an anti-CD47 antibody (WO2009/131453). When CD47 binds to the inhibitory immunoreceptor SIRP $\alpha$  expressed on monocytes, macrophages, dendritic cells 25 and neutrophils, SIRP $\alpha$  transmits an inhibitory signal that prevents destruction of cancer cells

by phagocytosis or other Fc-receptor-dependent cell destruction mechanisms of immune effector cells.

Tumour cells use up-regulation of CD47 as a mechanism to evade the anti-tumour immune response induced by a therapeutic antibody. Anti-CD47 or anti-SIRP $\alpha$  antibodies 5 block the inhibitory signalling generated via the CD47-SIRP $\alpha$  axis, resulting in an increase in ADCC and/or ADCP.

Most clinical research related to the CD47-SIRP $\alpha$  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 10 antibodies as anti-cancer therapeutics is growing, despite the fact that CD47 is also expressed on the surface of cells in most normal tissues.

Little research has been conducted on anti-cancer monotherapy or combination therapy using anti-SIRP $\alpha$  antibodies. The majority of the work on anti-SIRP $\alpha$  antibodies is mechanistic research regarding the CD47-SIRP $\alpha$  interaction and has been performed using 15 murine anti-SIRP $\alpha$  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). WO2015/138600 discloses murine anti-human SIRP $\alpha$  antibody KWAR23 and its chimeric Fab fragment, which increased the *in vitro* phagocytosis of i.a. cetuximab. Humanized KWAR23 with a human IgG<sub>1</sub> Fc part comprising a N297A mutation is disclosed 20 in WO2018/026600. WO2013/056352 discloses IgG<sub>4</sub> 29AM4-5 and other IgG<sub>4</sub> human anti-SIRP $\alpha$  antibodies. The IgG<sub>4</sub> 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.

SIRP $\alpha$  is a member of the family of signal regulatory proteins (SIRP), transmembrane 25 glycoproteins with extracellular Ig-like domains present on immune effector cells. The NH<sub>2</sub>-

terminal ligand binding domain of SIRP $\alpha$  is highly polymorphic (Takenaka *et al. Nature Immun.* 2007, 8(12), 1313-1323). However, this polymorphism does not influence binding to CD47 significantly. SIRP $\alpha$ <sub>BIT</sub> (v1) and SIRP $\alpha$ <sub>1</sub> (v2) are the two most common and most divergent (13 residues different) polymorphs (Hatherley *et al. J. Biol. Chem.* 2014, 289(14), 5 10024-10028). Other biochemically characterized human SIRP family members are SIRP $\beta$ <sub>1</sub>, and SIRP $\gamma$ .

SIRP $\beta$ <sub>1</sub> does not bind CD47 (van Beek *et al. J. Immunol.* 2005, 175 (12), 7781-7787, 7788-7789) and at least two SIRP $\beta$ <sub>1</sub> polymorphic variants are known, SIRP $\beta$ <sub>1v1</sub> (ENSP00000371018) and SIRP $\beta$ <sub>1v2</sub> (ENSP00000279477). Although the natural ligand of 10 SIRP $\beta$ <sub>1</sub> is yet unknown, *in vitro* studies using anti-SIRP $\beta$ <sub>1</sub> specific antibodies show that engagement of SIRP $\beta$ <sub>1</sub> promotes phagocytosis in macrophages by inducing the tyrosine phosphorylation of DAP12, 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).

15 SIRP $\gamma$  is expressed on T-cells and activated NK-cells and binds CD47 with a 10-fold lower affinity as compared to SIRP $\alpha$ . The CD47-SIRP $\gamma$  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, CD47-SIRP $\gamma$  interactions play a role in the transendothelial migration of T-cells (Stefanakis *et al. Blood* 20 2008, 112, 1280-1289).

The anti-SIRP $\alpha$  antibodies known in the art are less suitable for use in SIRP $\alpha$ -directed mono- or combination therapy, because they are either not specific for human SIRP $\alpha$ , or they are too specific. The prior art antibodies KW23, SE5A5, 29AM4-5 and 12C4 are not specific, as they also bind to human SIRP $\gamma$ . Binding to SIRP $\gamma$ , which is expressed on T-cells, 25 might negatively influence T-cell proliferation and recruitment. Other anti-SIRP $\alpha$  antibodies

have a too limited specificity, e.g. 1.23A mAb only recognizes the human SIRP $\alpha$  polymorphic variant SIRP $\alpha_1$  and not the variant SIRP $\alpha_{\text{BIT}}$ , which is predominant in at least the Caucasian population (X.W. Zhao *et al. PNAS* 2011, 108(45), 18342-18347).

Besides using anti-SIRP $\alpha$  antibodies to increase ADCC of a therapeutic antibody, these 5 antibodies may also be used to directly target SIRP $\alpha$ -expressing cancer types. Anti-SIRP $\alpha$  antibodies comprising wild-type human -Fc may be suitable to treat cancers expressing SIRP $\alpha$ , such as renal cell carcinoma and malignant melanoma, as murine anti-SIRP $\alpha$  antibodies having a functional Fc region slowed tumour formation in mice injected with Renca cells and B16BL6 melanoma cells, both expressing SIRP $\alpha$  (Yanagita *et al. JCI Insight* 10 2017, 2(1), e89140).

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

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## BRIEF DESCRIPTION OF THE PRESENT INVENTION

The present invention relates to antibodies against SIRP $\alpha$  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.

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## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Comparison of the ADCC measured in % cytotoxicity of trastuzumab (Tmab) alone, trastuzumab in combination with the murine 12C4 anti-SIRP $\alpha$  antibody (mu12C4), trastuzumab in combination with an antibody wherein murine 12C4 variable regions are 25 grafted onto the human IgG<sub>1</sub> constant region (12C4huIgG<sub>1</sub>), and trastuzumab in combination

with an antibody wherein murine 12C4 variable regions are grafted onto the human IgG<sub>1</sub> constant region comprising the amino acid substitutions L234A and L235A (12C4huIgG<sub>1</sub>LALA), measured on SKBR3 HER2-positive breast cancer cells using human neutrophils as effector cells.

5 Figure 2. Comparison of % ADCC relative to trastuzumab (set to 100%) of trastuzumab in combination with the anti-SIRP $\alpha$  antibodies 1-9 having a human IgG<sub>1</sub> constant region comprising the amino acid substitutions L234A and L235A, anti-SIRP $\alpha$  antibody 12C4huIgG<sub>1</sub>LALA (12C4LALA) and anti-CD47 antibody B6H12huIgG<sub>1</sub>LALA (B6H12LALA) on SKBR3 cells. Filled squares, (■), are the values measured with neutrophils 10 of donors having the SIRP $\alpha_{\text{BIT}}$  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-SIRP $\alpha$  antibodies 4, 7, 10, 14 in various concentrations (dose 15 response curves) having a human IgG<sub>1</sub> constant region comprising the amino acid substitutions L234A and L235A, and anti-SIRP $\alpha$  antibody 12C4huIgG<sub>1</sub>LALA (12C4LALA) on SKBR3 cells. Neutrophils of two donors ( $\Delta$ , ○) having the SIRP $\alpha_{\text{BIT}}$  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-SIRP $\alpha$  antibodies 4, 7, 10, 13, 14, 15 and 16 having a human IgG<sub>1</sub> constant region comprising the amino acid substitutions L234A and L235A, and anti-SIRP $\alpha$  antibody 12C4huIgG<sub>1</sub>LALA (12C4LALA) on SKBR3 cells. Neutrophils of donors having the SIRP $\alpha_{\text{BIT}}$  variant ( $\Delta$ ,  $\bigcirc$ ,  $\nabla$ ,  $\diamond$ ), having the SIRP $\alpha_1$  variant ( $\bigcirc$ ,  $\bullet$ ) and neutrophils of a donor which variant was not determined ( $\square$ ) were used. Columns are the average of the 25 donors.

## DETAILED DESCRIPTION OF THE PRESENT INVENTION

No approved therapeutics directed against SIRP $\alpha$  are available, although this target has been shown to play an important role in tumour immune evasion mechanisms. In addition, SIRP $\alpha$  is expressed on various malignant cells, rendering it a potential tumour associated 5 antigen.

The present invention relates to antagonistic anti-SIRP $\alpha$  antibodies which exhibit specific binding to the two predominant SIRP $\alpha$  polymorphic variants SIRP $\alpha$ <sub>B1T</sub> and SIRP $\alpha$ <sub>1</sub>, that do not bind to SIRP $\gamma$  and that increase the ADCC and/or ADCP of therapeutic antibodies.

The term “antibody” as used throughout the present specification refers to a monoclonal 10 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 IgG<sub>1</sub> or IgG<sub>2</sub> 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 IgG<sub>1</sub> 15 mAb. The antibody may have  $\kappa$  (kappa) or  $\lambda$  (lambda) light chains, preferably  $\kappa$  (kappa) light chains, i.e., a humanized or human IgG<sub>1</sub>- $\kappa$  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.

The terms “monoclonal antibody” and “mAb” as used herein refer to an antibody 20 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 25 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,

5 RNA may be isolated, reverse transcribed 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.

10 The term "antigen-binding fragment" as used throughout the present specification includes a Fab, Fab' or F(ab')<sub>2</sub> fragment, a single chain (sc) antibody, a scFv, a single domain (sd) antibody, a diabody, or a minibody.

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 15 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 20 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.

The CDRs may be determined using the approach of Kabat (in Kabat, E.A. *et al.*, 25 *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 5 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).

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 10 case, binding of an antagonistic anti-SIRP $\alpha$  antibody to SIRP $\alpha$  will either prevent binding of CD47 to SIRP $\alpha$  or disrupt the inhibitory signal that is triggered by the CD47-SIRP $\alpha$  binding.

Antagonistic anti-SIRP $\alpha$  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. Antagonistic 15 anti-SIRP $\alpha$  antibodies may also 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, e.g. a change in the three-dimensional shape of SIRP $\alpha$ . This change in the three-dimensional shape prevents (downstream) signalling upon binding to CD47. When SIRP $\alpha$  is bound at an allosteric site, CD47 may still be bound 20 by SIRP $\alpha$ , 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).

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 25 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 5 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.

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.

10 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.

15 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.

In particular, the present invention relates to an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof comprising heavy chain (HC) and light chain (LC) variable region 20 (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;
- 5 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;
- 10 g. 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;
- h. 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
- i. 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,

15 wherein the CDRs are determined according to Kabat numbering.

Preferably, the present invention relates to an anti-SIRP $\alpha$  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:

- 20 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;
- 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, CDR2 and CDR3 amino acid sequences of SEQ ID NO:10;
- e. 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;
- 5 f. 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;
- 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
- 10 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.

More preferably, the present invention relates to an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof comprising HCVR and LCVR CDRs selected from the group consisting of:

- 15 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;
- 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;
- 20 d. 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; and
- e. 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,

25 wherein the CDRs are determined according to Kabat numbering.

Even more preferably, the present invention relates to an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof comprising HCVR and LCVR CDRs selected from the group consisting of:

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

10 wherein the CDRs are determined according to Kabat numbering.

Most preferably, the present invention relates to an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof comprising HCVR and LCVR CDRs selected from the group consisting of:

- a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:7 and CDR1,  
15 CDR2 and CDR3 amino acid sequences of SEQ ID NO:8; and
- b. 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,

wherein the CDRs are determined according to Kabat numbering.

In a preferred embodiment, the present invention relates to an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof as defined hereinabove, wherein the antibody shows specific binding to both SIRP $\alpha_{\text{BIT}}$  and SIRP $\alpha_1$  and does not bind to SIRP $\gamma$ .

In a more preferred embodiment, the anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof specifically binds SIRP $\alpha_{\text{BIT}}$  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-SIRP $\alpha$  antibody or an antigen-binding fragment thereof binds SIRP $\alpha_1$  with a  $K_D$  below  $10^{-8}$  M.

In another more preferred embodiment, the anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof specifically binds SIRP $\alpha$ <sub>BIT</sub> and SIRP $\alpha$ <sub>1</sub> with a K<sub>D</sub> below 10<sup>-9</sup> M, wherein the K<sub>D</sub> is measured with SPR at 25°C.

In an even more preferred embodiment, the anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof specifically binds SIRP $\alpha$ <sub>BIT</sub> and SIRP $\alpha$ <sub>1</sub> with a K<sub>D</sub> below 10<sup>-10</sup> M. Preferably, the anti-SIRP $\alpha$  or an antigen-binding fragment thereof antibody specifically binds SIRP $\alpha$ <sub>BIT</sub> with a K<sub>D</sub> below 10<sup>-10</sup> M and SIRP $\alpha$ <sub>1</sub> with a K<sub>D</sub> below 10<sup>-11</sup> M. Typically, the anti-SIRP $\alpha$  antibody as defined hereinabove is a chimeric, humanized or human antibody. Preferably, the anti-SIRP $\alpha$  antibody is a humanized or human antibody. More preferably, the anti-SIRP $\alpha$  antibody is a humanized antibody. In a particular embodiment, the humanized anti-SIRP $\alpha$  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:30 and LCVR amino acid sequence of SEQ ID NO:31;
- 15 b. HCVR amino acid sequence of SEQ ID NO:32 and LCVR amino acid sequence of SEQ ID NO:33;
- c. HCVR amino acid sequence of SEQ ID NO:34 and LCVR amino acid sequence of SEQ ID NO:8;
- 20 d. HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:36;
- e. HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:37;
- f. HCVR amino acid sequence of SEQ ID NO:13 and LCVR amino acid sequence of SEQ ID NO:38; and

g. HCVR amino acid sequence of SEQ ID NO:13 and LCVR amino acid sequence of SEQ ID NO:37.

In a preferred embodiment, the humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof comprises HCVR amino acid sequence of SEQ ID NO:30 and LCVR amino acid sequence of SEQ ID NO:31.

In another preferred embodiment, the humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof comprises HCVR amino acid sequence of SEQ ID NO:32 and LCVR amino acid sequence of SEQ ID NO:33.

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:34 and LCVR amino acid sequence of SEQ ID NO:8.

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.

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.

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.

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.

Besides binding to both human (hu)SIRP $\alpha_{\text{BIT}}$  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.

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.

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

In one particular embodiment, the anti-SIRP $\alpha$  antibody according to the invention 15 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 20 ligation trigger phagocytosis, cytokine release, ADCC and/or ADCP, etc. Examples of these receptors are Fc $\gamma$  receptors, e.g. Fc $\gamma$ RI (CD64), Fc $\gamma$ RIIA (CD32), Fc $\gamma$ RIIIA (CD16a), Fc $\gamma$ RIIB (CD16b), Fc $\gamma$ RIIC and the Fc $\alpha$  receptor Fc $\alpha$ RI (CD89). The various natural antibody isotypes bind to these receptors. E.g. IgG<sub>1</sub> binds to Fc $\gamma$ RI, Fc $\gamma$ RIIA, Fc $\gamma$ RIIC, Fc $\gamma$ RIIIA, Fc $\gamma$ RIIB; IgG<sub>2</sub> binds to Fc $\gamma$ RIIA, Fc $\gamma$ RIIC, Fc $\gamma$ RIIIA; IgG<sub>3</sub> binds to Fc $\gamma$ RI, Fc $\gamma$ RIIA, Fc $\gamma$ RIIC, Fc $\gamma$ RIIIA, Fc $\gamma$ RIIB; IgG<sub>4</sub> binds to Fc $\gamma$ RI, Fc $\gamma$ RIIA, Fc $\gamma$ RIIC, Fc $\gamma$ RIIIA; 25 and IgA binds to Fc $\alpha$ RI.

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 IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> or IgG<sub>4</sub> isotype; the IgG<sub>1</sub>, IgG<sub>2</sub> or IgG<sub>4</sub> isotype is even more preferred. Most preferred is an anti-SIRP $\alpha$  antibody comprising an Fc region of the IgG<sub>1</sub> isotype.

5 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 $\alpha$  IgG<sub>1</sub> 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 10 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 IgG<sub>1</sub> 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.

15 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 20 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 Fc $\alpha$  or Fc $\gamma$  receptor in combination with a therapeutic antibody are 25 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-SIRP $\alpha$  antibody according to the invention is modified to reduce binding to activating Fc receptors present on human immune effector cells.

Therefore, the anti-SIRP $\alpha$  antibody according to the invention comprises a modified Fc region that exhibits reduced binding to or low affinity for a human Fc $\alpha$  or Fc $\gamma$  receptor. For instance, the IgG<sub>1</sub> binding to an Fc $\gamma$  receptor can be reduced by substituting one or more IgG<sub>1</sub> amino acids selected from the group consisting of L234, L235, G237, D265, D270, N297, A327, P328, and P329 (Eu numbering); the IgG<sub>2</sub> 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 IgG<sub>1</sub> Eu numbering) (Vafa *et al. Methods* 2014, 65, 114-126); the IgG<sub>3</sub> 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<sub>4</sub> binding can be reduced by introducing e.g. the amino acid substitutions S228P, F234A and L235A ((numbering analogue to IgG<sub>1</sub> Eu numbering) (Parekh *et al. mAbs* 2012, 4(3), 310-318). IgA binding to the Fc $\alpha$  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).

Preferably, the anti-SIRP $\alpha$  antibody according to the invention comprises a modified Fc region that exhibits reduced binding to or low affinity for a human Fc $\gamma$  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 IgG<sub>1</sub>, IgG<sub>2</sub> or IgG<sub>4</sub> isotype.

In a preferred embodiment, the anti-SIRP $\alpha$  antibody according to the invention comprises a modified human IgG<sub>1</sub> 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).

Preferably, the anti-SIRP $\alpha$  antibody comprises a modified Fc IgG<sub>1</sub> region, which does not comprise either amino acid substitution N297A or N297G. More preferably, the anti-SIRP $\alpha$  antibody comprises a modified Fc IgG<sub>1</sub> region, which does not comprise an amino acid substitution at position N297.

In one embodiment, the modified human IgG<sub>1</sub> 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.

In another embodiment, the modified human IgG<sub>1</sub> 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 IgG<sub>1</sub> region does not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgG<sub>1</sub> region does not comprise an amino acid substitution at position N297.

In a preferred embodiment, the modified human IgG<sub>1</sub> 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 IgG<sub>1</sub> region does not comprise either amino acid substitution N297A or N297G. More preferably, the modified Fc IgG<sub>1</sub> region does not comprise an amino acid substitution at position N297.

In another preferred embodiment, the anti-SIRP $\alpha$  antibody according to the invention comprises a modified human IgG<sub>1</sub> 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 IgG<sub>1</sub> region does not comprise either amino acid 5 substitution N297A or N297G. Even more preferably, the modified Fc IgG<sub>1</sub> region does not comprise an amino acid substitution at position N297.

The present invention further relates to a pharmaceutical composition comprising an anti-SIRP $\alpha$  antibody as described hereinabove and one or more pharmaceutically acceptable excipients. Typical pharmaceutical formulations of therapeutic proteins such as antibodies 10 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.

Typically, the pharmaceutical composition is provided in the form of a lyophilized cake. Suitable pharmaceutically acceptable excipients for inclusion into the pharmaceutical 15 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 20 formulations are selected for their ability to prevent protein denaturation during the freeze-drying process as well as during storage.

The present invention further relates to an anti-SIRP $\alpha$  antibody or pharmaceutical composition as described hereinabove for use as a medicament.

In one embodiment, the present invention relates to an anti-SIRP $\alpha$  antibody or pharmaceutical composition as described hereinabove for use in the treatment of human solid 25 tumours and haematological malignancies. The anti-SIRP $\alpha$  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).

In a second embodiment, the invention relates to an anti-SIRP $\alpha$  antibody comprising an Fc region that binds to activating Fc receptors present on human immune effector cells for use 5 in the treatment of SIRP $\alpha$ -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-SIRP $\alpha$  antibody comprising an Fc region of the IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> or IgG<sub>4</sub> isotype; the IgG<sub>1</sub>, IgG<sub>2</sub> or IgG<sub>4</sub> isotype is even more preferred. Most preferred is an anti-SIRP $\alpha$  antibody comprising an Fc 10 region of the IgG<sub>1</sub> isotype for use in the treatment of SIRP $\alpha$ -positive human solid tumours and haematological malignancies.

In a third embodiment, the present invention relates to an anti-SIRP $\alpha$  antibody 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 15 anti-cancer therapies. Suitable anti-cancer therapies are surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and immunotherapy. The anti-SIRP $\alpha$  antibody 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-SIRP $\alpha$  antibody 20 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.

Preferably, the present invention relates to an anti-SIRP $\alpha$  antibody or pharmaceutical composition as described hereinabove for use in the treatment of human solid tumours and 25 haematological malignancies in combination with the use of one or more other anti-cancer

therapeutics. In particular, the anti-SIRP $\alpha$  antibody 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.

Suitable anti-cancer therapeutics include chemotherapeutics, radiation therapeutics, 5 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 10 anthracyclines and bleomycins.

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

Suitable agents to be used as hormonal therapeutics include inhibitors of hormone 15 synthesis, such as aromatase inhibitors and GnRH analogues; and hormone receptor antagonists, such as selective oestrogen receptor modulators and antiandrogens.

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, 20 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 25 peptide derivative targeted therapeutics include proteasome inhibitors, such as bortezomib and carfilzomib.

Immunotherapeutic agents include agents that induce, enhance or suppress an immune response, such as cytokines (IL-2 and IFN- $\alpha$ ); 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 5 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.

Preferably, the invention relates to an anti-SIRP $\alpha$  antibody or pharmaceutical composition as described hereinabove for use in the treatment of human solid tumours and 10 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.

15 The term “therapeutic antibody” as used throughout the present specification refers to an antibody 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 20 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.

25 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.

The term “ADC” as used throughout the present specification refers to a cytotoxic drug conjugated to an antibody 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 5 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).

Typically, the therapeutic antibody for use in combination with an anti-SIRP $\alpha$  antibody according to the invention is a monospecific or bispecific antibody or antibody fragment 10 comprising at least one HCVR and LCVR binding to a target selected from the group consisting of annexin A1, B7H3, B7H4, CA6, CA9, CA15-3, CA19-9, CA27-29, CA125, CA242, CCR2, CCR5, 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 15 (e.g. EphA2 or EPhB3), endothelin B receptor (ETBR), FAP, FcRL5 (CD307), FGF, FGFR (e.g. FGFR3), FOLR1, GCC, GPNMB, HER2, HMW-MAA, integrin  $\alpha$  (e.g.  $\alpha$ v $\beta$ 3 and  $\alpha$ v $\beta$ 5), 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, 5T4 antigen (or TPBG, trophoblast glycoprotein), TF (tissue factor), Thomsen- 20 Friedenreich antigen (TF-Ag), Tag72, TNF, TNFR, TROP2, VEGF, VEGFR, and VLA.

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

Suitable therapeutic antibodies for use in combination with an anti-SIRP $\alpha$  antibody according to the invention include alemtuzumab, bevacizumab, cetuximab, panitumumab, 25 rituximab, and trastuzumab.

Suitable ADCs for use in combination with an anti-SIRP $\alpha$  antibody according to the invention include trastuzumab emtansine and brentuximab vedotin.

In a preferred embodiment, the present invention relates to an anti-SIRP $\alpha$  antibody as described hereinabove for the aforementioned use in combination with a therapeutic antibody 5 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.

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 10 human IgG, IgE, or IgA isotype comprise a human Fc region that binds to activating Fc receptors present on human immune effector cells.

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 IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, and IgG<sub>4</sub> antibodies. Even more preferred is a therapeutic 15 antibody of the IgG<sub>1</sub> or IgG<sub>2</sub> isotype. Most preferred is a therapeutic antibody of the IgG<sub>1</sub> isotype.

Preferably, the present invention relates to a humanized anti-SIRP $\alpha$  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,  
20 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;
- 5 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:13 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:14;
- 10 h. 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
- i. 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,

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 15 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-SIRP $\alpha$  antibody comprises a modified Fc region that exhibits reduced binding to a human Fc $\alpha$  or Fc $\gamma$  receptor, when compared to the same anti-SIRP $\alpha$  antibody comprising a wild-type Fc region.

In a preferred embodiment, the humanized anti-SIRP $\alpha$  antibody for use in the treatment 20 of human solid tumours and haematological malignancies in combination with the therapeutic antibody, comprises a modified human IgG<sub>1</sub> 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).

Preferably, the humanized anti-SIRP $\alpha$  antibody for use in the treatment of human solid 25 tumours and haematological malignancies in combination with the therapeutic antibody

comprises a modified Fc IgG<sub>1</sub> region, which does not comprise either amino acid substitution N297A or N297G. More preferably, the anti-SIRP $\alpha$  antibody comprises a modified Fc IgG<sub>1</sub> region, which does not comprise an amino acid substitution at position N297.

In one embodiment, the modified human IgG<sub>1</sub> 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.

In another embodiment, the humanized anti-SIRP $\alpha$  antibody for use in the treatment of human solid tumours and haematological malignancies in combination with the therapeutic antibody comprises a modified Fc IgG<sub>1</sub> 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 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 IgG<sub>1</sub> region does not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgG<sub>1</sub> region does not comprise an amino acid substitution at position N297.

In a preferred embodiment, the modified human IgG<sub>1</sub> 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 IgG<sub>1</sub> region does not comprise either amino acid substitution N297A or N297G. More preferably, the modified Fc IgG<sub>1</sub> region does not comprise an amino acid substitution at position N297.

In another preferred embodiment, the humanized anti-SIRP $\alpha$  antibody for use in the treatment of human solid tumours and haematological malignancies in combination with the therapeutic antibody comprises a modified human IgG<sub>1</sub> 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 IgG<sub>1</sub> region does not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgG<sub>1</sub> region does not comprise an amino acid substitution at position N297.

5        In a preferred embodiment, the humanized anti-SIRP $\alpha$  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 10 L235A, and HCVR and LCVR CDRs selected from the group consisting of:

15        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;

20        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;

25        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;

30        d.      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; and

35        e.      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.

In a second 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 and LCVR CDRs selected from the group consisting of:

25        a.      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;

- b. 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; and
- c. 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.

5 In a third 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 and LCVR CDRs selected from the group consisting of:

- a. 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; and
- b. 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.

10 In a fourth 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

- 15 a. HCVR amino acid sequence of SEQ ID NO:30 and LCVR amino acid sequence of SEQ ID NO:31;
- b. HCVR amino acid sequence of SEQ ID NO:32 and LCVR amino acid sequence of SEQ ID NO:33;
- c. HCVR amino acid sequence of SEQ ID NO:34 and LCVR amino acid sequence of SEQ ID NO:8;
- 20 d. HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:36;
- e. HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:37;

f. HCVR amino acid sequence of SEQ ID NO:13 and LCVR amino acid sequence of SEQ ID NO:38; or

g. HCVR amino acid sequence of SEQ ID NO:13 and LCVR amino acid sequence of SEQ ID NO:37.

5 In one 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:30 and LCVR amino acid sequence of SEQ ID NO:31. More preferably, the modified Fc IgG<sub>1</sub> region does not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgG<sub>1</sub> region does not comprise an amino acid substitution at position N297.

10 In 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:32 and LCVR amino acid sequence of SEQ ID NO:33.

15 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:34 and LCVR amino acid sequence of SEQ ID NO:8.

20 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.

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.

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 5 and L235A, and HCVR amino acid sequence of SEQ ID NO:13 and LCVR amino acid sequence of SEQ ID NO:38.

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 10 sequence of SEQ ID NO:37.

More preferably, the humanized anti-SIRP $\alpha$  antibodies as defined hereinabove for use as defined hereinabove comprising an Fc region comprising the amino acid substitutions L234A and L235A, the modified Fc IgG<sub>1</sub> region do not comprise either amino acid substitution N297A or N297G. Even more preferably, the modified Fc IgG<sub>1</sub> region does not 15 comprise an amino acid substitution at position N297.

The anti-SIRP $\alpha$  antibodies comprising a modified Fc region that exhibits reduced binding to a human Fc $\alpha$  or Fc $\gamma$  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 20 either SIRP $\alpha$ <sub>B1T</sub> or SIRP $\alpha$ <sub>1</sub>. 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.

## EXAMPLES

### Immunization protocol and selection

Rabbits were repeatedly immunized with a mixture of peptides representing the extra cellular domain region of human (hu)SIRP $\alpha$ <sub>BIT</sub>, human (hu)SIRP $\alpha$ <sub>1</sub> and cynomolgus

5 (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; 10 subsequently the specific binding huSIRP $\alpha$ <sub>BIT</sub> and huSIRP $\alpha$ <sub>1</sub>, to cySIRP $\alpha$  and to an anti-Fc antibody was determined. Suitable supernatants were those binding to both huSIRP $\alpha$ <sub>BIT</sub> and huSIRP $\alpha$ <sub>1</sub> and to cySIRP $\alpha$ . After a hit picking step binding to mouse (mu) SIRP $\alpha$  and to huSIRP $\beta$ <sub>1v1</sub>, huSIRP $\beta$ <sub>1v2</sub> and huSIRP $\gamma$  (as anti-targets) was measured. In addition, the binding to SIRP $\alpha$ <sub>BIT</sub> and SIRP $\alpha$ <sub>1</sub>-over expressing CHO cells was determined. Binding to parental 15 CHO cells was applied as a control assay.

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 IgG<sub>1</sub> HC-LALA format SEQ ID NO:27), respectively.

20 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 $\alpha$ <sub>1</sub>, huSIRP $\alpha$ <sub>BIT</sub>, cySIRP $\alpha$ , muSIRP $\alpha$ , huSIRP $\beta$ <sub>1v1</sub>/ $\beta$ <sub>1v2</sub>/ $\gamma$ ; cell binding assays: huSIRP $\alpha$ <sub>1</sub>, huSIRP $\alpha$ <sub>BIT</sub>). 25

**Transient expression of antibodies****a) Preparation of cDNA constructs and expression vectors**

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 5 10-14), and at the C-terminus to the constant domain of a human IgG<sub>1</sub> HC LALA according to SEQ ID NO:27. The HCVR amino acid sequences of antibodies 12C4huIgG<sub>1</sub>LALA, 12C4huIgG<sub>1</sub> or 29AM4-5huIgG<sub>1</sub>LALA 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 IgG<sub>1</sub> HC LALA according to SEQ ID NO:27 or a wild type human IgG<sub>1</sub> HC (SEQ ID 10 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 12C4huIgG<sub>1</sub>LALA, 12C4huIgG<sub>1</sub> and 29AM4-5huIgG<sub>1</sub>LALA) 15 to the LCVR of antibodies 1-16, 12C4huIgG<sub>1</sub>LALA and 12C4huIgG<sub>1</sub> and 29AM4-5huIgG<sub>1</sub>LALA at the N-terminus and at the C-terminus to a human IgG<sub>1</sub> κ 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
29AM4-5huIgG <sub>1</sub> LALA	SEQ ID NO:19	SEQ ID NO:20
12C4huIgG <sub>1</sub> LALA	SEQ ID NO:21	SEQ ID NO:22
12C4huIgG <sub>1</sub>	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

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 5 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).

c) Transient expression in mammalian cells

Commercially available Expi293F cells (Thermo Fisher) were transfected with the expression vectors using the ExpiFectamine transfection agent according to the 10 manufacturer's instructions as follows:  $75 \times 10^7$  cells were seeded in 300 mL FortiCHO medium, 300  $\mu$ g of the expression vector was combined with 800  $\mu$ l of ExpiFectamine transfection 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 15 harvest over PES bottle filters/ MF 75 filters (Nalgene).

### Antibody binding and specificity

#### *Experimental*

ELISA assay: Solutions of huSIRP $\alpha_1$ , huSIRP $\alpha_{\text{BIT}}$ , huSIRP $\beta_{1v1}$ , huSIRP $\beta_{1v2}$ , huSIRP $\gamma$  20 and cySIRP $\alpha$  in phosphate buffered saline (PBS) were each added to a multiple well black polystyrene plate for ELISA and allowed to adhere 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 25 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')<sub>2</sub>:horse radish peroxidase (HRP) in buffer was added to the wells and incubated at RT for 1 h. 3,3',5,5'-Tetramethylbenzidine (TMB) was added and after sufficient colour development HCl was added. Absorbance was read at 450 nm/620 nm.

5        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) 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) 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 calculated using the Biacore T200 evaluation software (v3.1).

10      15      20      25

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 (1x PBS (LONZA) containing 0.2% v/w BSA (Sigma-Aldrich, St. Louis, MO) and 0.02% v/w NaN<sub>3</sub> (Sigma-Aldrich), followed by the addition of a concentration range of each primary mAb (50  $\mu$ L/well) diluted in ice-cold FACS buffer. After an incubation 5 time of 30 min at 4°C, the cells were washed three times with ice-cold FACS buffer and 50  $\mu$ L/well secondary mAb (AffiniPure F(ab')<sub>2</sub> 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 10 and resuspended in 150  $\mu$ L FACS buffer. Fluorescence intensities were determined by flow cytometry (BD FACSVerse, Franklin Lakes, NJ) and indicated as the 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<sub>50</sub> values were calculated as the concentration in  $\mu$ g/mL that gives a response half way between bottom and 15 top of the curve, when using a 4-parameter logistic fit.

15

### *Results*

ELISA assay: The EC<sub>50</sub> values for binding to huSIRP $\alpha$ <sub>1</sub>, huSIRP $\alpha$ <sub>BIT</sub>, huSIRP $\beta$ <sub>1</sub>, huSIRP $\beta$ <sub>1v2</sub>, huSIRP $\gamma$ , cySIRP $\alpha$  obtained with ELISA for antibodies 1-9 and reference 20 antibodies are summarized in Table 2. All antibodies bind to huSIRP $\alpha$ <sub>1</sub> and to huSIRP $\alpha$ <sub>BIT</sub>, Antibodies 29AM4-5huIgG<sub>1</sub>LALA and 12C4huIgG<sub>1</sub>LALA, bind to huSIRP $\beta$ <sub>1v1</sub>, huSIRP $\beta$ <sub>1v2</sub>, and huSIRP $\gamma$ . The antibodies 2-6, 8 and 9 show a low affinity for huSIRP $\beta$ <sub>1v1</sub> and for huSIRP $\gamma$ . Antibody 7 binds to huSIRP $\beta$ <sub>1v1</sub>, but has low affinity for huSIRP $\beta$ <sub>1v2</sub> and huSIRP $\gamma$ . Antibody 1 binds to huSIRP $\beta$ <sub>1v2</sub> and huSIRP $\gamma$ .

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

Antibody	huSIRP $\alpha_1$	huSIRP $\alpha_{\text{BIT}}$	huSIRP $\beta_{1v1}$	huSIRP $\beta_{1v2}$	huSIRP $\gamma$	cySIRP $\alpha$
	EC <sub>50</sub> (ng/ml)	EC <sub>50</sub> (ng/ml)	EC <sub>50</sub> (ng/ml)	EC <sub>50</sub> (ng/ml)	EC <sub>50</sub> (ng/ml)	EC <sub>50</sub> (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

\*huIgG<sub>1</sub>LALAEC<sub>50</sub> values > 100,000 have been adjusted to 100,000.

5        SPR assay: The K<sub>D</sub> values for binding to huSIRP $\alpha_1$ , huSIRP $\alpha_{\text{BIT}}$  and huSIRP $\gamma$  of antibodies 4, 7, 10-14 in comparison with reference antibodies KWAR23, huIgG<sub>1</sub>12C4LALA 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 $\alpha_{\text{BIT}}$ , and do not bind to huSIRP $\gamma$ . All reference antibodies do bind to huSIRP $\gamma$ .

Table 3 SPR data ( $K_D$  in M)

Antibody	$K_D$ (huSIRP $\alpha_{BIT}$ )	$K_D$ (huSIRP $\alpha_1$ )	$K_D$ (huSIRP $\gamma$ )
KWAR23 mouse IgG2a	<1.0E-11 <sup>1</sup>	<1.0E-11	<1.0E-11
KWAR23 huIgG <sub>1</sub> LALA	<1.0E-11 <sup>1</sup>	1.1E-11	<1.0E-11
12C4huIgG <sub>1</sub> LALA	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 <sup>2</sup>
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

<sup>1</sup> <1.0E-11:  $K_D$  is outside the range which means high affinity

<sup>2</sup> N: No specific binding found

5 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<sub>50</sub> 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.

Table 4 Flow Cytometry data

Antibody	U937 cells (SIRP $\alpha$ <sub>BIT</sub> ) EC <sub>50</sub> (μg/mL)	ExpiCHO-S (huSIRP $\alpha$ <sub>1</sub> ) EC <sub>50</sub> (μg/mL)	ExpiCHO-S (huSIRP $\alpha$ <sub>BIT</sub> ) EC <sub>50</sub> (μg/mL)	ExpiCHO-S (cySIRP $\alpha$ ) EC <sub>50</sub> (μg/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 huIgG <sub>1</sub> LALA	0.25	-	-	-
12C4huIgG <sub>1</sub> LALA	0.19	-	-	-
KWAR23 huIgG <sub>1</sub> LALA	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

Antibody	U937 cells (SIRP $\alpha$ <sub>BIT</sub> ) EC <sub>50</sub> (μg/mL)	ExpiCHO-S (huSIRP $\alpha$ <sub>1</sub> ) EC <sub>50</sub> (μg/mL)	ExpiCHO-S (huSIRP $\alpha$ <sub>BIT</sub> ) EC <sub>50</sub> (μg/mL)	ExpiCHO-S (cySIRP $\alpha$ ) EC <sub>50</sub> (μg/mL)
15	0.16	-	-	-
16	0.23	-	-	-

- value not determined

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

#### Experimental

5 CHO cells transfected with either SIRP $\alpha$ <sub>1</sub> or SIRP $\alpha$ <sub>BIT</sub> or parental CHO cells as control were seeded in 20 μl cell medium in a well plate with clear bottom and incubated overnight. Antibodies 1-9, 29AM4-5huIgG<sub>1</sub>LALA or 12C4huIgG<sub>1</sub>LALA 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 10 wash buffer. Fluorescence was determined using a screening system (CellInsight®, Thermo Scientific®) and total fluorescence per cell was determined.

#### Results

Antibodies 29AM4-5huIgG<sub>1</sub>LALA, 12C4huIgG<sub>1</sub>LALA, 3 and 7 block binding of CD47 to both CHO cells expressing huSIRP $\alpha$ <sub>1</sub> and CHO cells expressing huSIRP $\alpha$ <sub>BIT</sub> completely, 15 antibodies 1, 2, 4-6, 8 and 9 do neither block binding of CD47 to CHO cells expressing huSIRP $\alpha$ <sub>1</sub> nor to CHO cells expressing huSIRP $\alpha$ <sub>BIT</sub>.

### ADCC assay

Neutrophils of donors homozygous for either SIRP $\alpha$ <sub>1</sub> or SIRP $\alpha$ <sub>BIT</sub> were isolated and 20 cultured according to the method in Chao *et al.* PNAS 2011, 108(45), 18342-18347. ADCC

was determined using the  $^{51}\text{Cr}$  release assay or the non-radioactive Europium TDA (EuTDA) cytotoxicity assay (DELFIA, PerkinElmer). SKBR3 cells were used as target cells and labelled with 100  $\mu\text{Ci}$   $^{51}\text{Cr}$  (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 5 washes with PBS,  $5 \times 10^3$  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%  $\text{CO}_2$  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 10 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  $[(\text{experimental release} - \text{spontaneous release}) / (\text{total release} - \text{spontaneous release})] \times 100\%$ . All conditions were measured in duplicate and/or triplicate.

15

#### *ADCC data 12C4huIgG<sub>1</sub>LALA versus 12C4IgG<sub>1</sub>*

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 20 (mu12C4). Trastuzumab in combination with an antibody wherein 12C4 variable regions are grafted onto a human IgG<sub>1</sub> constant region (12C4huIgG<sub>1</sub>) shows similar % cytotoxicity as compared to trastuzumab alone at low concentrations of 12C4huIgG<sub>1</sub>. At higher concentrations 12C4huIgG<sub>1</sub>, a decrease in % cytotoxicity is observed. Trastuzumab in combination with an antibody wherein 12C4 variable regions are grafted onto a human IgG<sub>1</sub> 25 constant region comprising amino acid substitutions L234A and L235A (12C4huIgG<sub>1</sub>LALA)

shows increased % cytotoxicity compared to the % cytotoxicity of trastuzumab alone, and increased % cytotoxicity compared to the combination of 12C4huIgG<sub>1</sub> and trastuzumab.

*ADCC data*

5 Figure 2 compares the % ADCC by human neutrophils relative to trastuzumab (set to 100%) in the presence of antibody 1-9 having a human IgG<sub>1</sub> constant region comprising amino acid substitutions L234A and L235A (LALA) in combination with trastuzumab in comparison with 12C4huIgG<sub>1</sub>LALA. B6H12IgG<sub>1</sub>LALA, having the VR of a murine anti-CD47 antibody and a human IgG<sub>1</sub> constant region comprising amino acid substitutions 10 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 $\alpha_{\text{BIT}}$  variant (homozygous for SIRP $\alpha_{\text{BIT}}$ ), 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 15 antibodies 1, 2, 4, 5, 7 and 8 the average ADCC increase was enhanced even more than the 12C4huIgG<sub>1</sub>LALA-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 12C4huIgG<sub>1</sub>LALA.

Figure 3 compares the % ADCC by human neutrophils in the presence of various 20 concentrations of chimeric antibodies 4 and 7 and humanized antibodies 10 and 14 having a human IgG<sub>1</sub> 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 12C4huIgG<sub>1</sub>LALA. Neutrophils of two donors homozygous for SIRP $\alpha_{\text{BIT}}$  were used. Even at low concentrations antibodies 4, 7, 10 and 14 25 increase ADCC. The ADCC increase is concentration dependent.

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 12C4huIgG<sub>1</sub>LALA. 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 12C4huIgG<sub>1</sub>LALA 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)**

5    SEQ ID NO:1 (HC VR 1)

1       QSVEESGGRL VTPGTPLTLT CTVSGIDLSS YAMSWVRQAP GKGLEWIGII  
51      SSGGITYYYAS WAKGRFTISK TSTTVDLKIP SPTTEDTATY FCARSLWAAS  
101     NYYMALWGPG TLTVVSS

10    SEQ ID NO:2 (LC VR 1)

1       AIKMTQTPAS VSAAVGGTVS INCQASEDIE SYLAWYQQKP GQPPKLLIYR  
51      ASTLASGVSS RFKGSGSGTQ FTLTISDLES ADAATYYCLG DYYSSSGDTG  
101     AFGGGTEVVV K

15    SEQ ID NO:3 (HC VR 2)

1       QSVEESGGRL VTPGTPLTLT CTVSGFSLSN YAMHWVRQAP GKGLEWIGII  
51      YTGGATSYAT WAKGQFTISK TSTTVDLKIT SPTTEDTATY FCARGDRDGY  
101     AYFNIWGPGT LTVVSL

20    SEQ ID NO:4 (LC VR 2)

1       QIVMTQTPFS VSAVVGTVT IKCQASHNIG SWLAWYQQKP GQRPKLLIYD  
51      ASTLASGVSS RFKGSGSGTE FTLTISGVES ADAATYYCQQ GYGISYVHNV  
101     FGGGTEVVVK

## SEQ ID NO:5 (HC VR 3)

1 QSVEESGGRL VTPGTPLTLA CTVSGFSLIS YYISWVRQAP EKGLEYIGII  
 51 NIGGGASYAS WAKGRFTISK TSTTVDLKIT SPTPEDTATY FCAMSYGMDT  
 101 GAFNIWGP GT LTVSL

5

## SEQ ID NO:6 (LC VR 3)

1 AQVLTQTPAS VSAAVGGTVT ISQSSESVY KNNFLSWYQQ KPGKPPKLLI  
 51 YGASTLASGV PSRFKGSGSG TQFTLTISDL ESDDAATYFC QGGYRTDIYP  
 101 FGGGTEVVVK

10

## SEQ ID NO:7 (HC VR 4)

1 QSVEESGGRL GTPGTPLTLT CTVSGFSLSS YVMGWFRQAP GKGLEYIGII  
 51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SPTTEDTATY FCARVGPLGV  
 101 DYFNIWGP GT LTVSL

15

## SEQ ID NO:8 (LC VR 4)

1 DIVMTQTPSS VEAvggtvt IKCQAGQSIN SYLAWYQQKP GQRPKLLIYY  
 51 ASTLESGVPS RFKGSGSGTD YTTLTISDLES ADAATYYCQS WHYISRSYAF  
 101 GGGTEVVVK

20

## SEQ ID NO:9 (HC VR 5)

1 QSVEESGGRL VTPGTPLTLT CTVSGFSLSS YVMGWFRQAA GKGLEYIGYI  
 51 NADGSPYYAT WVNGRFTISK TPTTMDLKIN SPTTEDTATY FCARVGPLGV  
 101 DYFNIWGP GT LTVSL

## SEQ ID NO:10 (LC VR 5)

1 DIVMTQTPAS VEAvggvt IKCQASQSI RYLTWYQQKP GQRPKLLIYY  
 51 ASTLESGVPS RFEGRGSGTD YTLLTISDLES ADAATYYCQS YYYISRTYAF  
 101 GGGTEV VVK

5

## SEQ ID NO:11 (HC VR 6)

1 QSVEESGGRL VTPGTPLTLT CTVSGIDLSS YTMTWVRQAP GKGLEWIGI  
 51 YAGGSTAYAS WAKGRFTISK TSTTVDLKIT SPTTEDTATY FCARSSSDGY  
 101 DYFNIWGPgt LTVVS L

10

## SEQ ID NO:12 (LC VR 6)

1 GVVMTQTPSS VSAAVGGVT INCQASQSI SWLAWYQQKP GQPPKLLIYQ  
 51 ASKLASGVPS RFSGRGSGTH FTLLTISDVQS DDAATYYCQQ TVTAASNVDNA  
 101 FGGGTEVVVK

15

## SEQ ID NO:13 (HC VR 7)

1 RSVEESGGRL VTPGTPLTLT CTVSGFSLSS HGISWVRQAP GKGLYEIGT  
 51 GTGVITYFAS WAKGRFTGSK TSTTVDLKIT SPTTEDTATY FCARGSAWND  
 101 PFDPWGPgtL VTVSS

20

## SEQ ID NO:14 (LC VR 7)

1 ALVMTQTPAS VSAAVGGVT TKCQASQSVY GNNDLAWYQH KPGQPPKLLI  
 51 YLASTLATGV PSRFSGSGSG TQFTLTITGV QSDDAATYYC LGGGDDEADN  
 101 VFGGGTEVVV K

## SEQ ID NO:15 (HC VR 8)

1 QSLEESGGRL VTPGTPLTLT CTASGVDSL YAMGWVRQAP GKGLEWIGII  
 51 YAGGSTSYAT WAKGRFTISK TSTTMDLKMT SPTTEDTATY FCARHRSDGY  
 101 DYFHLWGP GT LTVSL

5

## SEQ ID NO:16 (LC VR 8)

1 AIDMTQTPAS VSEPVGGTVT IKCQASQSIS SWLAWYQQKP GQRPKLLIYD  
 51 ASKLASGVPS RFSGSGSGTE FTLTISGVQS DDAAAYYCQQ GYAVSYVENI  
 101 FGGGTEVVVK

10

## SEQ ID NO:17 (HC VR 9)

1 QSMEESGGRL VTPGTPLTLT CTASGFSLN YGVSWVRQAP GKGLEWIGII  
 51 YGGSDITAYA SWAKGRFTIS KTSTTVDLTI TSPTTEDTAT YFCAKSYTNG  
 101 MDYYNIWGPG TLTVSL

15

## SEQ ID NO:18 (LC VR 9)

1 AFDLTQTPSS VEAPVGGTVI IKCQASQSIS SYLAWYQQKP GQPPKLLIY  
 51 ASTLASGVSS RFKGSGSETQ FPLTISDLES ADAATYYCQS YYGSRSNVFG  
 101 GGTEVVVK

20

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

1 EVQLVESGGG LVQPGGSLRL SCAASGFNIS YYFIHWVRQA PGKGLEWVAS  
 51 VYSSFGYTY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARFT  
 101 FPGLFDGFFG AYLGSLDYWG QGTLTVSS

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

1 DIQMTQSPSS LSASVGDRV TTCRASQSVS SAVAWYQQKP GKAPKLLIYS  
 51 ASSLYSGVPS RFSGSRSGTD FTLTISSLQP EDFATYYCQQ AVNWVGALVT  
 101 FGQGTKVEIK

5

## SEQ ID NO:21 (HC VR 12C4)

1 EVKLEESGGG LMQP<sup>G</sup>GS<sup>M</sup>KL SCVASGFTFS NYWMNWVRQS PEKGLEWVAE  
 51 IRLKSNNYAT HYAESVKGRF TISRDDSKSS VYLQMNNLRA EDTGIYYCIR  
 101 DYDYDAYFDY WGQGTTLTVS S

10

## SEQ ID NO:22 (LC VR 12C4)

1 DIVLTQSPAS LAVSLGQRAT ISCRASKSVS TSGYN<sup>M</sup>YWY QQKPGQPPKL  
 51 LIYL<sup>A</sup>SNLES GVPARFSGSG SGTDFTLN<sup>I</sup>H PVEEDAATY YCQHSGELPY  
 101 TFGGGTKLEI K

15

## SEQ ID NO:23 (HC VR KWAR23 )

1 EVQLQQSGAE LVKPGASVKL SCTASGFNIK DYYIH<sup>W</sup>VQQR TEQGLEWIGR  
 51 IDPEDGETKY APKFQDKATI TADTSSNTAY LHLSSLTSED TAVYYCARWG  
 101 AYWGQGTLVT VSS

20

## SEQ ID NO:24 (LC VR KWAR23)

1 QIVLTQSPAI MSASPGEKVT LTCSASSSVS SSYLYWYQQK PGSSPKLWIY  
 51 STSNLASGVP ARFSGSGSGT SYSLTISSME AEDAASYFCH QWSSYPRTFG  
 101 AGTKLELK

25

SEQ ID NO:25 (human IgG<sub>1</sub> antibody HC constant region)

1 ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV  
 51 HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP  
 101 KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS  
 5 151 HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK  
 201 EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC  
 251 LVKGFYPSDI AVEWESNGQP ENNYKTPPPV LDSDGSFFLY SKLTVDKSRW  
 301 QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

10 SEQ ID NO:26 (human IgG<sub>1</sub> antibody LC κ constant region)

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

15 SEQ ID NO:27 (human IgG<sub>1</sub> 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  
 20 151 HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK  
 201 EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC  
 251 LVKGFYPSDI AVEWESNGQP ENNYKTPPPV 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)

5 1 MACPGFLWAL VISTCLEFSMA

SEQ ID NO:30 (HC VR 10)

1 KVEESGGGLV QPGGSLRLSC AASGFSLSSY VMGWVRQAPG KGLEWVSIIS  
51 SSGSPYYASW VNGRFTISKD NSEGMVYLQM NSLRAEDTAV YYCARVGPLG  
10 101 VDYFNIWGQG TTDTVSS

SEQ ID NO:31 (LC VR 10)

1 DIVMTQSPDS LAVSLGERAT INCQAGQSIN SYLAWYQQKP GQPPKLLIYY  
51 ASTLESGVPD RFSGSGSGTD FTLLTISSLQA EDVAVYYCQS WHYISRSYAF  
15 101 GGGTKLEIK

SEQ ID NO:32 (HC VR 11)

1 EVKVEESGGG LVQPGGSLRL SCAASGFSLS SYVMGWVRQA PGKGLEWVSI  
51 ISSSGSPYYA SWVNGRFTIS KTSTTMDLQM NSLRAEDTAV YYCARVGPLG  
20 101 VDYFNIWGQG TTDTVSS

SEQ ID NO:33 (LC VR 11)

1 DIQMTQSPSS LSASVGDRVT ITCQAGQSIN SYLAWYQQKP GKVPKLLIYY  
51 ASTLESGVPS RFSGSGSGTD FTLLTISSLQP EDVATYYCQS WHYISRSYAF  
25 101 GQGTKVEIK

## SEQ ID NO:34 (HC VR 12)

1 VQLVESGGRL VQPGTPLTLS CTVSGFSLSS YVMGWFRQAP GKGLYEIGII  
51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SLRSEDTATY FCARVGPLGV  
101 DYFNIWGP GT LTVSS

5

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

1 RQLVESGGGL VQPGGSLRLS CTASGFSLSS HGISWVRQAP GKGLYEIGTI  
51 GTGVITYFAS WAKGRFTGSK TSSTAYMELS SLRSEDTAVY FCARGSAWND  
101 PFDPWGQGTL VTVSS

10

## SEQ ID NO:36 (LC VR 13)

1 AIQMTQSPSS LSASVGDRVT ITCQASQSVY GNNDLAWYQQ KPGKAPKLLI  
51 YLASTLATGV PSRFSGSGSG TDFTLTISL QPEDFATYYC LGGGDDEADN  
101 VFGGGTTKVEI K

15

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

1 DIEMTQSPSS VSASVGDRVT LTCQASQSVY GNNDLAWYQQ KPGQAPKLLI  
51 YLASTLATGV PSRFSGSGSG TDFTLTISL QPEDFATYYC LGGGDDEADN  
101 VFGGGTTKVEI K

20

## SEQ ID NO:38 (LC VR 15)

1 ELVMTQSPSS LSASVGDRVT ITCQASQSVY GNNDLAWYQQ KPGEAPKLLI  
51 YLASTLATGV PSRFSGSGSG TDFTLTISGL QSEDFATYYC LGGGDDEADN  
101 VFGQGTTKVEI K

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

1 MGWTLVFLFL LSVTAGVHS

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

5 1 MVSSAQFLGL LLLCFQGTRC

## CLAIMS

1. An anti-SIRP $\alpha$  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:

- 5 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;
- 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;
- 10 d. 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;
- e. 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;
- 15 f. 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;
- 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;

20 wherein the CDRs are determined according to Kabat numbering.

2. The antibody according to claim 1, which is chimeric, humanized or human.
3. The antibody according to claim 2, comprising HCVR and LCVR CDRs selected from the group consisting of

- a. 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; and
- b. 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;

5 wherein the antibody is humanized.

4. The humanized antibody according to claim 3, comprising
  - a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:7, CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:8, HCVR amino acid sequence of SEQ ID NO:30 and LCVR amino acid sequence of SEQ ID NO:31;
  - b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:7, CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:8, HCVR amino acid sequence of SEQ ID NO:32 and LCVR amino acid sequence of SEQ ID NO:33;
  - c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:7, CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:8, HCVR amino acid sequence of SEQ ID NO:34 and LCVR amino acid sequence of SEQ ID NO:8;
  - d. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:13, CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:14, HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:36;
  - e. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:13, CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:14 and HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:37;
  - f. HCVR amino acid sequence of SEQ ID NO:13, CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:14 and LCVR amino acid sequence of SEQ ID NO:38; or

g. HCVR amino acid sequence of SEQ ID NO:13, CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:14 and LCVR amino acid sequence of SEQ ID NO:37.

5. The anti-SIRP $\alpha$  antibody according to any one of claims 1 to 4 comprising a modified Fc region that exhibits reduced binding to a human Fc $\alpha$  or Fc $\gamma$  receptor compared to the same anti-SIRP $\alpha$  antibody comprising a wild-type Fc region.
6. The anti-SIRP $\alpha$  antibody according to any one of claims 1 to 5 comprising a modified human IgG<sub>1</sub> 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, 10 A327, P328, and P329 according to Eu numbering.
7. The anti-SIRP $\alpha$  antibody according to claim 6 comprising the amino acid substitutions L234A and L235A, L234E and L235A, L234A, L235A and P329A or L234A, L235A and P329G.
8. The anti-SIRP $\alpha$  antibody according to claim 7 comprising the amino acid substitutions L234A and L235A or L234E and L235A.
- 15 9. A pharmaceutical composition comprising the anti-SIRP $\alpha$  antibody according to any one of claims 1 to 8 and one or more pharmaceutically acceptable excipients.
10. The anti-SIRP $\alpha$  antibody according to any one of claims 1 to 8 or the pharmaceutical composition according to claim 9 for use as a medicament.
- 20 11. The anti-SIRP $\alpha$  antibody or the pharmaceutical composition for use according to claim 9 for use in the treatment of human solid tumours and haematological malignancies.
12. A combination of the anti-SIRP $\alpha$  antibody or the pharmaceutical composition for use according to claim 10 with one or more other anti-cancer therapeutics for use in the treatment of human solid tumours and haematological malignancies.

13. The combination for use according to claim 12, wherein the one or more anti-cancer therapeutics are targeted therapeutics or immunotherapeutic agents.
14. The combination for use according to claim 12, wherein the targeted therapeutic is a therapeutic antibody or an antibody-drug conjugate.
- 5 15. The combination for use according to claim 14, wherein the therapeutic antibody is 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.

FIGURE 1

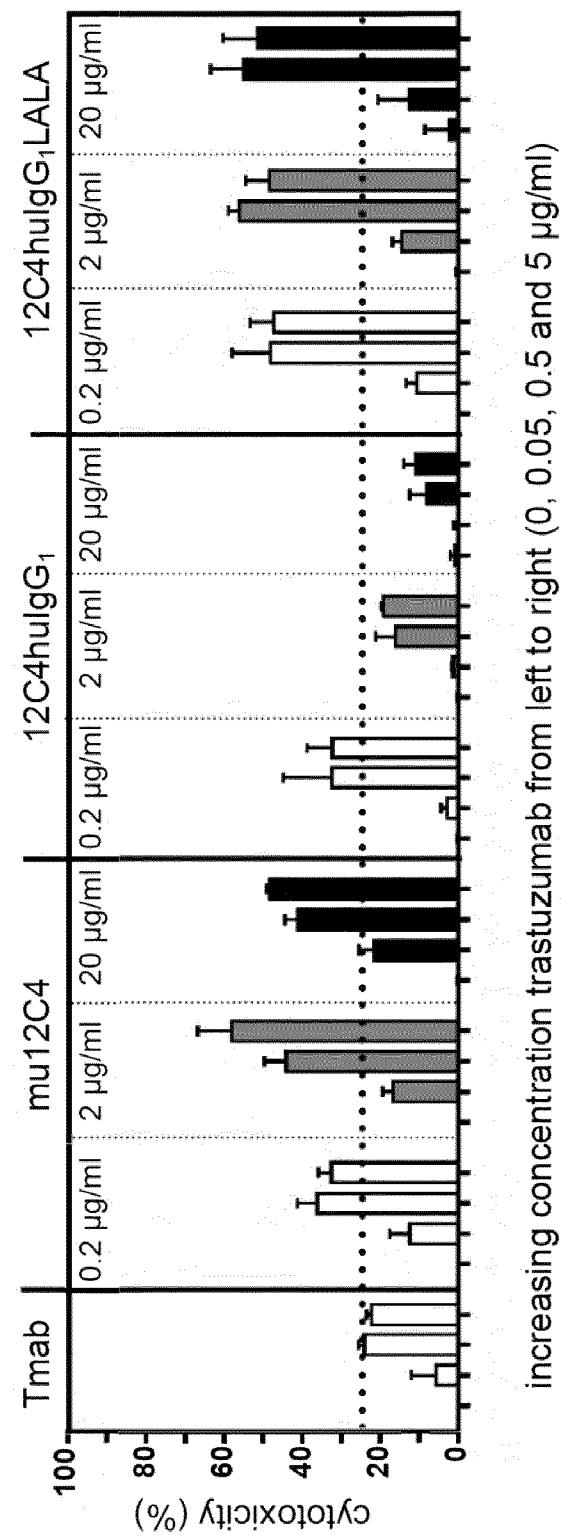


FIGURE 2

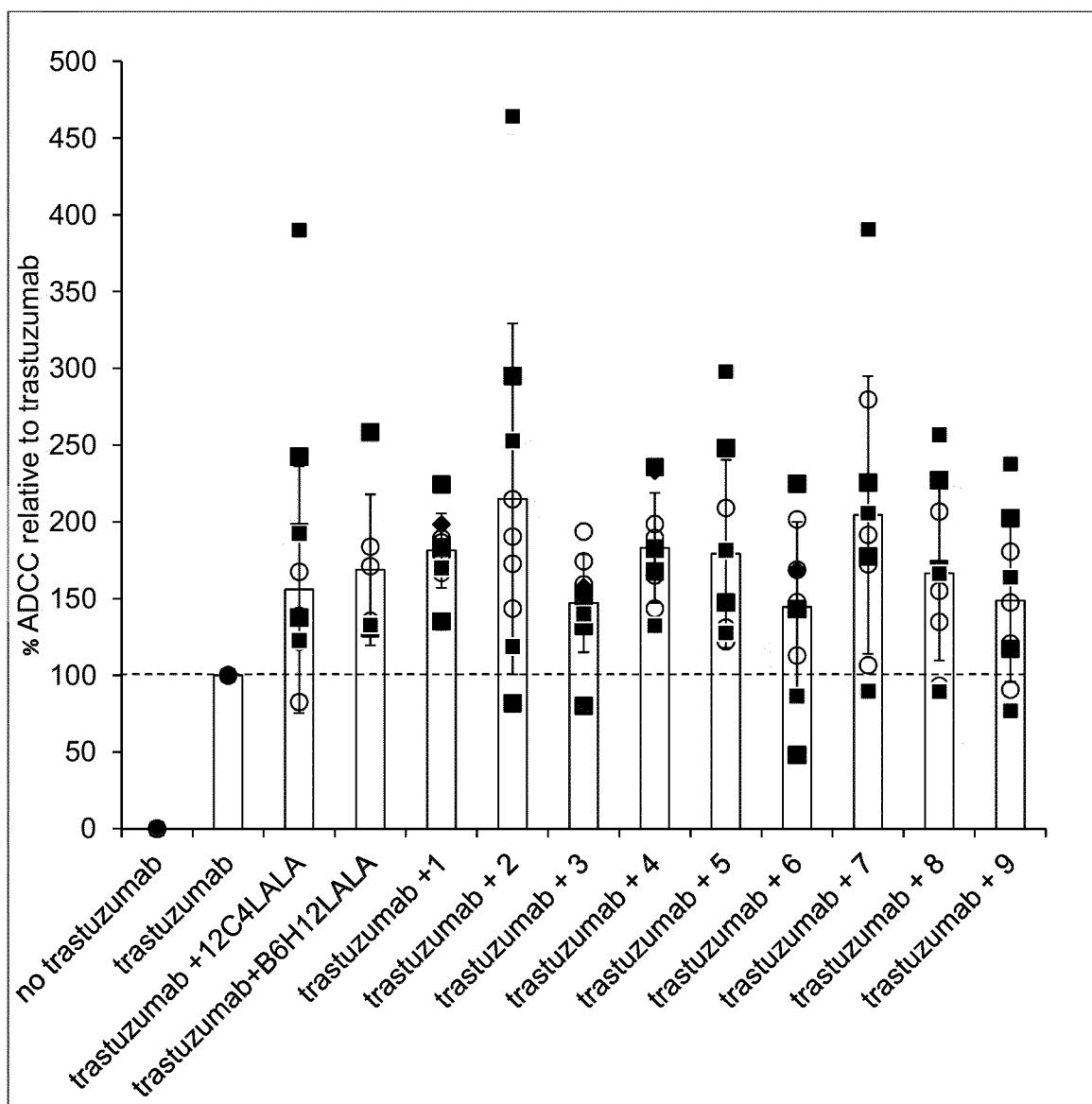


FIGURE 3

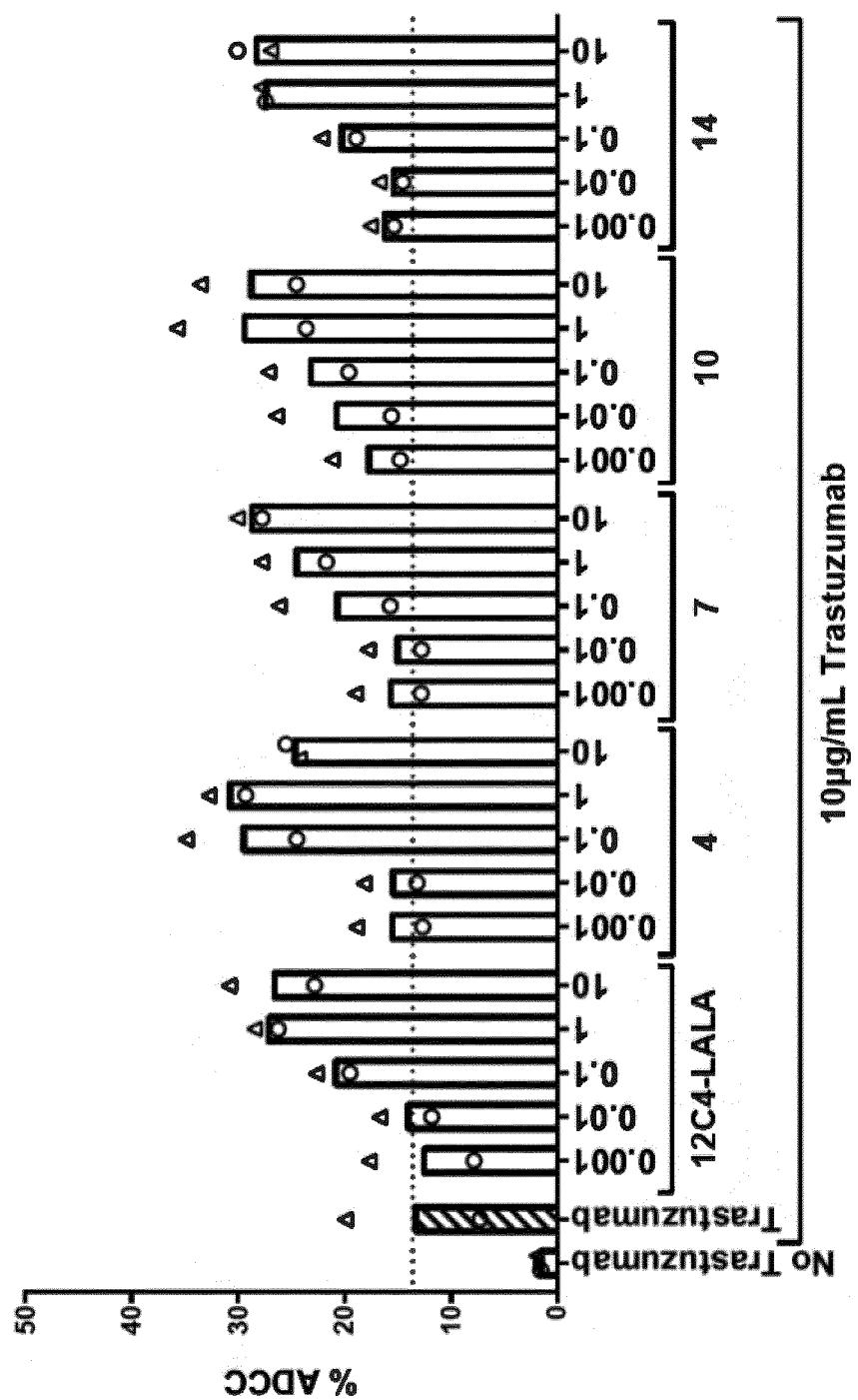


FIGURE 4

