

## HUMANIZED ANTI-SIRP $\alpha$ ANTIBODIES

### FIELD OF THE INVENTION

5       The present invention relates to humanized antibodies against SIRP $\alpha$  and the use of these antibodies in the treatment of cancer, optionally in combination with anti-cancer therapeutics.

### BACKGROUND OF THE INVENTION

10       Since the late 1990s, therapeutic antibodies that recognize antigens on tumour cells have been available for the treatment of cancer. These therapeutic antibodies can act upon malignant cells via different pathways. The signalling pathways triggered by binding of the antibody to its target on malignant cells result in inhibition of cell proliferation or in apoptosis. The Fc region of the therapeutic antibody can trigger complement dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and/or antibody-dependent cellular  
15 phagocytosis (ADCP). Another possible mechanism may be the antibody-dependent induction of a T-cell (CD8<sup>+</sup> and/or CD4<sup>+</sup>) anti-tumour response (antibody-dependent antigen presentation (ADAP); DiLillo and Ravetch *Cell* 2015, 161(5), 1035-1045; Bournazos and Ravetch *Immunity* 2017, 47(2), 224-233). This occurs via the Fc receptors expressed on antigen presenting cells, such as for example dendritic cells. However, therapeutic antibodies are often not effective  
20 enough as monotherapy. One option to improve the efficacy of therapeutic antibodies is through improving ADCC and/or ADCP. This has for example 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 glycosylation of the Fc region (Hayes *et al. J. Inflamm. Res.* 2016, 9, 209–219).

25       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  $\alpha$  (SIRP $\alpha$ ) or an anti-CD47 antibody (WO2009/131453). When CD47 – which has been found to be upregulated in and/or on at least several human tumour types – binds to the inhibitory immunoreceptor SIRP $\alpha$  expressed on monocytes, macrophages, dendritic cells and  
30 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. One of the mechanisms through which anti-CD47 or anti-SIRP $\alpha$  antibodies are hypothesized to act is through blocking of the inhibitory signalling generated via the CD47-SIRP $\alpha$  axis, resulting in an increase in ADCC and/or ADCP and/or ADAP (Tjeng *et al. Proc*

*Natl Acad Sci USA* 2013, 110(27), 11103-11108; Liu *et al. Nature Med.* 2015, 21(10), 1209-1215).

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; Advani *et al. N. Engl. J. Med.* 2018, 379(18), 1711-1721). Research regarding anti-CD47 antibodies as anti-cancer therapeutics is growing, despite the fact that CD47 is widely expressed on the surface of cells in most normal tissues.

No clinical 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 murine anti-SIRP $\alpha$  antibodies; e.g. murine 12C4 and 1.23A were reported to increase 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 were reported to increase cetuximab-mediated phagocytosis *in vitro*. Humanized KWAR23 with a human IgG<sub>1</sub> Fc part comprising a N297A mutation is disclosed 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 acute myeloid leukaemia (AML) cells injected into the right femur of NOD scid gamma (NSG) mice. WO2017/178653 discloses chimeric anti-SIRP $\alpha$  antibody HEFLB which binds to SIRP $\alpha_1$  and SIRP $\alpha_{BIT}$ , but not to SIRP $\gamma$ . However, although the antibody retains binding to SIRP $\alpha_{BIT}$  upon humanization, it no longer binds to SIRP $\alpha_1$ . Since for instance 51.3% of the Caucasians have at least 1 allele of SIRP $\alpha_1$ , their immune cells are (at least partly when heterozygous) unresponsive to an antibody only binding SIRP $\alpha_{BIT}$  (Treffers *et al. Eur J Immunol.* 2018, 48(2), 344-354). WO2018/057669 discloses humanized chicken anti-SIRP $\alpha$  antibodies against domain 1 of human SIRP $\alpha_1$  and/or human SIRP $\alpha_{BIT}$ . WO2018/107058 discloses that mouse anti-SIRP $\alpha$  antibodies 3F9 and 9C2 do not bind to SIRP $\beta_{1v1}$  and they concluded that these antibodies are therefore SIRP $\alpha$ -specific, with equilibrium binding constants of  $1.0 \times 10^{-8}$  and  $8.0 \times 10^{-8}$  M, respectively. WO2018/190719 discloses humanized anti-SIRP $\alpha$  antibodies that also bind to human SIRP $\gamma$ , but that do not bind to human SIRP $\beta_1$ .

Human SIRP $\alpha$  is highly polymorphic in its NH<sub>2</sub>-terminal ligand binding domain (Takenaka *et al. Nature Immun.* 2007, 8(12), 1313-1323): SIRP $\alpha_{BIT}$  (v1) and SIRP $\alpha_1$  (v2) are the two most common and most divergent (13 residues different) polymorphs and their affinities

for CD47 are very similar (Hatherley *et al. J. Biol. Chem.* 2014, 289(14), 10024-10028; Treffers *et al. Eur J Immunol.* 2018, 48(2), 344-354). Other biochemically characterized human SIRP family members are SIRP $\beta_1$ , which does not bind CD47 and has at least two polymorphic variants (SIRP $\beta_{1v1}$  and SIRP $\beta_{1v2}$ ), and SIRP $\gamma$ , which is expressed on T-cells and activated NK-  
5 cells and binds CD47 with an approximately 10-fold lower affinity as compared to SIRP $\alpha$  (van Beek *et al. J Immunol.* 2005, 175(12), 7781-7). The CD47-SIRP $\gamma$  interaction is involved in the contact between antigen-presenting cells and T-cells, respectively, 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  
10 (Stefanidakis *et al. Blood* 2008, 112, 1280-1289).

A disadvantage of the anti-SIRP $\alpha$  antibodies known in the art is that they are either (i) not specific for human SIRP $\alpha$ , because they bind to other human SIRP family members such as human SIRP $\gamma$ , thereby possibly resulting in undesirable side effects, or (ii) they are too limited in their specificity, only binding to one of the SIRP $\alpha$  allelic variants – for example SIRP $\alpha_{BIT}$  or  
15 SIRP $\alpha_1$  – thereby making them less suitable for mono- or combination therapy, since part of the human population has a SIRP $\alpha$  allelic variant to which the anti-SIRP $\alpha$  antibody does not bind. For example, prior art antibodies KWAR23, SE5A5, 29AM4-5 and 12C4 are not specific, as they also bind to human SIRP $\gamma$ , which might negatively influence T-cell proliferation and recruitment. Conversely, 1.23A mAb for example is too specific and only recognizes the human  
20 SIRP $\alpha$  polymorphic variant SIRP $\alpha_1$  and not the variant SIRP $\alpha_{BIT}$ , which is predominant in at least the Caucasian population (Zhao *et al. PNAS* 2011, 108(45), 18342-18347; Treffers *et al. Eur J Immunol.* 2018, 48(2), 344-354). Also humanized antibody HEFLB disclosed in WO2017/178653 is too specific, as this antibody does not bind to SIRP $\alpha_1$  (SPR measurements; see Example section) and does not promote anti-tumour activity of immune effector cells from  
25 SIRP $\alpha_1$  carriers, even when one allele of SIRP $\alpha_{BIT}$  is present (Figure 3).

Only later published WO2018/210793 discloses several antagonistic chimeric anti-SIRP $\alpha$  antibodies which exhibit specific binding to the two predominant SIRP $\alpha$  polymorphic variants SIRP $\alpha_{BIT}$  and SIRP $\alpha_1$ , that do not bind to SIRP $\gamma$  and that increase the ADCC of therapeutic antibodies (antibodies 2-9). In Table 1 of WO2018/210793, humanized variants for two of the  
30 chimeric antibodies are disclosed (antibodies 10-16).

In conclusion, there is still a need in the art for further and improved anti-SIRP $\alpha$  antibodies that are useful in anti-cancer therapy either alone or in combination with a further therapeutic anti-cancer antibody. More in particular, a need remains for antagonistic anti-SIRP $\alpha$  antibodies which have no, low, or reduced binding to human SIRP $\gamma$ , thereby reducing the risk

for undesirable side effects, and which anti-SIRP $\alpha$  antibodies bind to both human SIRP $\alpha_1$  and human SIRP $\alpha_{BIT}$  polymorphic variants, to make them suitable for a large part of the human population, including SIRP $\alpha_1$ /SIRP $\alpha_{BIT}$  heterozygotes, SIRP $\alpha_{BIT}$  homozygotes and SIRP $\alpha_1$  homozygotes. There is a need for anti-SIRP $\alpha$  antibodies with these characteristics and which  
5 reduce inhibitory, i.e. SHP-1 and/or SHP-2-mediated, SIRP $\alpha$  signalling. Such antibodies are suitable for use in anti-cancer therapy either alone or, preferably, in combination with a therapeutic anti-cancer antibody.

### BRIEF DESCRIPTION OF THE INVENTION

10 The present invention relates to humanized antibodies against SIRP $\alpha$  that are suitable for use in anti-cancer therapy alone or, preferably, in combination with anti-cancer therapy such as a therapeutic anti-cancer antibody.

In a first aspect, the present invention relates to a humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof, comprising heavy chain complementarity determining  
15 regions (HCDR) and light chain complementarity determining regions (LCDR) HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the antibody or the antigen-binding fragment thereof comprises:

- a. HCDR1 comprising SEQ ID NO:36;
- b. HCDR2 comprising SEQ ID NO:44;
- 20 c. HCDR3 comprising SEQ ID NO:45;
- d. LCDR1 comprising SEQ ID NO:39;
- e. LCDR2 comprising SEQ ID NO:40; and
- f. LCDR3 comprising SEQ ID NO:41.

In a preferred embodiment, the anti-SIRP $\alpha$  antibody or the antigen-binding fragment  
25 thereof has one or more properties from the group consisting of: (a) the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human SIRP $\alpha_1$  with a binding affinity of at least  $10^{-10}$  M, preferably at least  $10^{-11}$  M, as analysed by surface plasmon resonance (SPR) (preferably BiaCore<sup>TM</sup>) at 25°C using human SIRP $\alpha_1$  extracellular domain as shown in SEQ ID NO:51; (b) the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human  
30 SIRP $\alpha_{BIT}$  with a binding affinity of at least  $10^{-10}$  M, preferably at least  $10^{-11}$  M, as analysed by SPR (preferably BiaCore<sup>TM</sup>) at 25°C using human SIRP $\alpha_{BIT}$  extracellular domain as shown in SEQ ID NO:52; (c) the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds cynomolgus monkey SIRP $\alpha$  with a binding affinity of at least  $10^{-8}$  M, preferably at least  $10^{-9}$  M, as analysed by SPR (preferably BiaCore<sup>TM</sup>) at 25°C using cynomolgus SIRP $\alpha$  extracellular

domain as shown in SEQ ID NO:56; (d) the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof does not bind human SIRP $\gamma$  as measured by T-cell binding using flow cytometry, preferably using fluorescence-activated cell sorting (FACS) staining; (e) the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof does not bind human SIRP $\gamma$  as analysed by SPR (preferably BiaCore<sup>TM</sup>) at 25°C using human SIRP $\gamma$  extracellular domain as shown in SEQ ID NO:55; and (f) the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof is not immunogenic as determined by IL-2 enzyme-linked immunosorbent spot (ELISpot) and/or T-cell proliferation assay.

In a preferred embodiment, the humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof: (a) binds human SIRP $\alpha_1$  with a binding affinity of at least  $10^{-10}$  M, preferably at least  $10^{-11}$  M, as analysed by SPR (preferably BiaCore<sup>TM</sup>) at 25°C using human SIRP $\alpha_1$  extracellular domain as shown in SEQ ID NO:51; (b) binds human SIRP $\alpha_{BIT}$  with a binding affinity of at least  $10^{-10}$  M, preferably at least  $10^{-11}$  M, as analysed by SPR (preferably BiaCore<sup>TM</sup>) at 25°C using human SIRP $\alpha_{BIT}$  extracellular domain as shown in SEQ ID NO:52; (c) blocks CD47 binding to SIRP $\alpha_1$  and SIRP $\alpha_{BIT}$ , preferably as analysed by dissociation from captured CD47 by SPR (preferably BiaCore<sup>TM</sup>), more preferably as described in Example 6; and (d) does not bind human SIRP $\gamma$  as measured by T-cell flow cytometry, preferably fluorescence-activated cell sorting (FACS) staining.

In a preferred embodiment, the invention relates to a humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof, wherein: (a) the heavy chain variable domain of the antibody comprises 4 heavy chain framework regions, HFR1 to HFR4, and 3 complementarity determining regions HCDR1 to HCDR3 that are operably linked in the order HFR1-HCDR1-HFR2-HCDR2-HFR3-HCDR3-HFR4, wherein each of the heavy chain framework regions has at least 90% amino acid identity with the framework amino acid sequence of SEQ ID NO:8 or wherein HFR1 to HFR4 differ from SEQ ID NO:8 in one or more of the amino acid substitutions as defined in Tables 8 to 11; and (b) the light chain variable domain of the antibody comprises 4 light chain framework regions, LFR1 to LFR4, and 3 complementarity determining regions LCDR1 to LCDR3 that are operably linked in the order LFR1-LCDR1-LFR2-LCDR2-LFR3-LCDR3-LFR4, wherein each of the light chain framework regions has at least 90% amino acid identity with the framework amino acid sequence of SEQ ID NO:9, or wherein LFR1, LFR2 and/or LFR4 differ from SEQ ID NO:9 in one or more amino acid substitutions as defined in Tables 12 to 14.

In a preferred embodiment, a humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to the invention, comprises a heavy chain variable region (HCVR)

amino acid sequence and a light chain variable region (LCVR), wherein the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof comprises the HCVR amino acid sequence of SEQ ID NO:8 and the LCVR amino acid sequence of SEQ ID NO:9.

In a preferred embodiment, a humanized anti-SIRP $\alpha$  antibody according to the invention,  
5 comprises 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, preferably a reduction of at least 10, 20, 30, 40, 50, 60, 70, 80, 90% or a reduction of 100%. In a preferred embodiment, a modified human IgG<sub>1</sub> Fc region comprises an amino acid substitution 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. More preferably, a modified human IgG<sub>1</sub> Fc region comprises amino acid substitutions: L234A and L235A; L234E and L235A; L234A, L235A and P329A; or L234A, L235A and P329G. More preferably the antibody comprises the amino acid substitutions L234A and L235A; or, L234E and L235A. Most preferably, the antibody comprises amino acid substitutions L234A and L235A. In a preferred embodiment,  
15 the human IgG Fc region does not comprise other amino acid substitutions.

In a second aspect, the present invention relates to a pharmaceutical composition comprising a humanized anti-SIRP $\alpha$  antibody, or an antigen-binding fragment thereof, according to the invention and a pharmaceutically acceptable excipient.

In a third aspect, the present invention relates to a humanized anti-SIRP $\alpha$  antibody, or an  
20 antigen-binding fragment thereof, according to the invention, or a pharmaceutical composition according to the invention for use as a medicament.

In a fourth aspect, the present invention relates to a humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to the invention or a pharmaceutical composition according to the invention for use in the treatment of a cancer, wherein the treatment further  
25 comprises administration of a therapeutic antibody, wherein the cancer preferably is a human solid tumour or a haematological malignancy. Preferably, the therapeutic antibody is directed against a membrane-bound target on the surface of tumour cells and comprises a human Fc region that binds to activating Fc receptors present on human immune effector cells.

In a preferred embodiment, the human solid tumour is selected from the group consisting  
30 of (HER2-positive) breast cancer, (EGFR-positive) colon carcinoma, (GD2-positive) neuroblastoma, melanoma, osteosarcoma, (CD20-positive) B-cell lymphomas, (CD38-positive) multiple myeloma (CD52-positive) lymphoma, (CD33-positive) acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML), chronic lymphatic leukaemia (CLL), acute lymphoblastic leukaemia (ALL), non-Hodgkin's lymphoma (NHL), including follicular

lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL), hepatocellular carcinoma, multiple myeloma (MM), bladder cancer, gastric cancer, ovarian cancer, head and neck cancer, pancreatic cancer, renal carcinoma, prostate cancer, hepatocellular carcinoma and lung cancer. In a preferred embodiment, the treatment comprises administration of a further anti-cancer  
5 therapeutic compound, such as for example a targeted therapeutic, preferably an immunotherapeutic agent.

In a fifth aspect, the present invention relates to a nucleic acid molecule comprising a nucleotide sequence encoding a humanized anti-SIRP $\alpha$  antibody or an antigen binding fragment thereof according to the invention, wherein preferably the nucleic acid molecule comprises a  
10 nucleotide sequence encoding at least one of the HCVR and the LCVR of the antibody, and wherein preferably the coding nucleotide sequence is operably linked to regulatory sequences for expression of the coding nucleotide sequence in a host cell.

In a sixth aspect, the present invention relates to a host cell comprising the nucleic acid molecule according to the invention.

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## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Binding of antibodies to SIRP $\gamma$  expressing human CD3<sup>+</sup> T-cells by flow cytometry. Data is depicted as mean fluorescence intensity (MFI) (Figure 1a) and percentage (%) of positive cells (Figure 1b). Symbols represent measurements from two healthy  
20 individuals.

Figure 2. 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 grafted onto the human IgG<sub>1</sub> constant region (12C4huIgG<sub>1</sub>), and trastuzumab in combination with an  
25 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; also indicated as 12C4-LALA herein), measured using SKBR3 HER2-positive breast cancer cells as target cells and human neutrophils as effector cells. Neutrophils were isolated from human donors carrying two SIRP $\alpha$ <sub>BIT</sub> alleles. Increasing concentrations of trastuzumab were  
30 employed: 0, 0.05, 0.5 and 5  $\mu$ g/ml, respectively, as well as increasing concentrations of each anti-SIRP $\alpha$  antibody (0.2, 2 and 20  $\mu$ g/ml, respectively).

Figure 3. Neutrophil-mediated ADCC towards trastuzumab (Tmab; 10  $\mu$ g/ml) opsonized SKBR3 cells in combination with prior art based anti-SIRP $\alpha$  antibodies at various concentrations ( $\mu$ g/ml; dose response curves). Neutrophils were isolated from heterozygous

human donors carrying one  $SIRP\alpha_1$  and one  $SIRP\alpha_{BIT}$  allele. Each individual neutrophil donor is indicated by a symbol. Columns are the average of all donors. As controls, untreated cells and cells treated with 10  $\mu\text{g/ml}$  trastuzumab were used. Data is normalized for response to trastuzumab (set to 100%). Experiment was performed with neutrophils stimulated O/N with G-CSF and  $IFN\gamma$ . Cytotoxicity was measured with DELFIA cytotoxicity assay.

Figure 4. Neutrophil-mediated ADCC towards trastuzumab (Tmab; 10  $\mu\text{g/ml}$ ) opsonized SKBR3 cells in combination with anti- $SIRP\alpha$  antibodies 1-6 of the invention, having a human  $IgG_1$  constant region comprising the amino acid substitutions L234A and L235A, at various concentrations ( $\mu\text{g/ml}$ ; dose response curves). Neutrophils were isolated from human donors carrying one  $SIRP\alpha_1$  and one  $SIRP\alpha_{BIT}$  allele. Each individual neutrophil donor is indicated by a symbol. Columns are the average of all donors. As controls, untreated cells and cells treated with 10  $\mu\text{g/ml}$  trastuzumab were used. Data is normalized for response to trastuzumab (set to 100%). Experiment was performed with neutrophils stimulated O/N with G-CSF and  $IFN\gamma$ . Cytotoxicity was measured with DELFIA cytotoxicity assay.

Figure 5. Neutrophil-mediated ADCC towards trastuzumab (Tmab; 10  $\mu\text{g/ml}$ ) opsonized SKBR3 cells in combination with anti- $SIRP\alpha$  antibodies 7-13 of the invention, having a human  $IgG_1$  constant region comprising the amino acid substitutions L234A and L235A, at various concentrations ( $\mu\text{g/ml}$ ; dose response curves). Neutrophils were isolated from human donors carrying one  $SIRP\alpha_1$  and one  $SIRP\alpha_{BIT}$  allele. Each individual neutrophil donor is indicated by a symbol. Columns are the average of all donors. Data is normalized for response to trastuzumab (set to 100%). Experiment was performed with neutrophils stimulated O/N with G-CSF and  $IFN\gamma$ . Cytotoxicity was measured with DELFIA cytotoxicity assay.

Figure 6. Neutrophil-mediated ADCC towards trastuzumab (Tmab; 10  $\mu\text{g/ml}$ ) opsonized SKBR3 cells in combination with prior art based anti- $SIRP\alpha$  antibodies at various concentrations ( $\mu\text{g/ml}$ ; dose response curves). Neutrophils were isolated from human donors carrying two  $SIRP\alpha_1$  alleles. Each individual neutrophil donor is indicated by a symbol. Columns are the average of all donors. As controls, untreated cells and cells treated with 10  $\mu\text{g/ml}$  trastuzumab were used. Data is normalized for response to trastuzumab (set to 100%). Experiment was performed with neutrophils stimulated for 100 min with GM-CSF and cytotoxicity was measured as  $^{51}\text{Cr}$  release assay.

Figure 7. Neutrophil-mediated ADCC towards trastuzumab (Tmab; 10  $\mu\text{g/ml}$ ) opsonized SKBR3 cells in combination with anti- $SIRP\alpha$  antibodies 7-13 of the invention, having a human  $IgG_1$  constant region comprising the amino acid substitutions L234A and L235A, at various concentrations ( $\mu\text{g/ml}$ ; dose response curves). Neutrophils were isolated from human donors

carrying two SIRP $\alpha_1$  alleles. Each individual neutrophil donor is indicated by a symbol. Columns are the average of all donors. As controls, untreated cells and cells treated with 10  $\mu$ g/ml trastuzumab were used. Data is normalized for response to trastuzumab (set to 100%). Experiment was performed with neutrophils stimulated for 100 min with GM-CSF and cytotoxicity was measured as  $^{51}\text{Cr}$  release assay.

Figure 8. Neutrophil-mediated ADCC towards trastuzumab (Tmab; 10 $\mu$ g/ml) opsonized SKBR3 cells in combination with prior art based anti-SIRP $\alpha$  antibodies at various concentrations ( $\mu$ g/ml; dose response curves). Neutrophils were isolated from human donors carrying two SIRP $\alpha_{\text{BIT}}$  alleles. Each individual neutrophil donor is indicated by a symbol. Columns are the average of all donors. As controls, untreated cells and cells treated with 10  $\mu$ g/ml trastuzumab were used. Data is normalized for response to trastuzumab (set to 100%). Experiment was performed with neutrophils stimulated for 100 min with GM-CSF and cytotoxicity was measured as  $^{51}\text{Cr}$  release assay.

Figure 9. Neutrophil-mediated ADCC towards trastuzumab (Tmab; 10 $\mu$ g/ml) opsonized SKBR3 cells in combination with anti-SIRP $\alpha$  antibodies 1-6 of the invention, having a human IgG $_1$  constant region comprising the amino acid substitutions L234A and L235A, at various concentrations ( $\mu$ g/ml; dose response curves). Neutrophils were isolated from human donors carrying two SIRP $\alpha_{\text{BIT}}$  alleles. Each individual neutrophil donor is indicated by a symbol. Columns are the average of all donors. As controls, untreated cells and cells treated with 10  $\mu$ g/ml trastuzumab were used. Data is normalized for response to trastuzumab (set to 100%). Experiment was performed with neutrophils stimulated for 100 min with GM-CSF and cytotoxicity was measured as  $^{51}\text{Cr}$  release assay.

Figure 10a-d. Binding of indicated anti-SIRP $\alpha$  antibodies to granulocytes (left panels), CD14 $^+$  monocytes (middle panels) and CD3 $^+$  T-cells (right panels) as determined by flow cytometry in whole blood of a representative healthy heterozygous SIRP $\alpha_1$ /SIRP $\alpha_{\text{BIT}}$  donor. The relevant isotype control for each anti-SIRP $\alpha$  antibody is provided in each graph. Data is depicted as median fluorescence intensity (MFI).

Figure 11. Binding of indicated anti-SIRP $\alpha$  antibodies to SIRP $\gamma$  expressing human CD3 $^+$  T-cells isolated from buffy coats of a healthy donor as determined by flow cytometry. The relevant isotype control for each anti-SIRP $\alpha$  antibody is provided in each graph. Data is depicted as mean fluorescence intensity (MFI).

Figure 12a. SHP-1 recruitment to SIRP $\alpha$  as measured by relative luminescence units (RLU) using Jurkat SIRP $\alpha_{\text{BIT}}$  signalling cells with Jurkat E6.1 cells as CD47 ligand cells, in absence or presence of a concentration range of antibody 6 or an isotype control. The % of

maximum signal was determined as follows: (RLU/RLU of maximal stimulation (no antibody) \*100). Results are shown as mean +/- SEM of two independent experiments.

Figure 12b. Efficacy levels of indicated anti-SIRP $\alpha$  antibodies at 3.3  $\mu$ g/ml for inhibiting SIRP $\alpha_{BIT}$  signalling as measured with Jurkat SIRP $\alpha_{BIT}$  signalling cells and Jurkat E6.1 cells as CD47 ligand cells. Efficacy levels were calculated as followed: 100% - '% of maximum signal' of 3.3  $\mu$ g/ml compound value. Results are shown as mean +/- SEM of two independent experiments.

Figure 13a-b. Neutrophil-mediated ADCC towards trastuzumab (10  $\mu$ g/ml) opsonized SKBR3 cells in combination with anti-SIRP $\alpha$  antibody 6 of the invention and indicated reference antibodies. Neutrophils were isolated from human donors carrying two SIRP $\alpha_{BIT}$  alleles, carrying two SIRP $\alpha_1$  alleles or carrying one SIRP $\alpha_{BIT}$  and one SIRP $\alpha_1$  allele. Columns are the average of all donors +/- SEM. As controls, untreated cells and cells treated with 10  $\mu$ g/ml trastuzumab were used. For each antibody, dose response curves were made at 10, 1, 0.1 and 0.01  $\mu$ g/ml (from left to right). Antibody 6 and reference antibodies are shown in panel (a); Isotype controls are shown in panel (b). Data is normalized for response to trastuzumab (set to 100%). Experiment was performed as indicated in Experimental section 10.

Figure 14a-b. Neutrophil-mediated ADCC towards cetuximab (5  $\mu$ g/ml) opsonized A431 cells in combination with anti-SIRP $\alpha$  antibody 6 of the invention and indicated reference antibodies. Neutrophils were isolated from human donors carrying two SIRP $\alpha_{BIT}$  alleles, carrying two SIRP $\alpha_1$  alleles or carrying one SIRP $\alpha_{BIT}$  and one SIRP $\alpha_1$  allele. Columns are the average of all donors. As controls, untreated cells and cells treated with 5  $\mu$ g/ml cetuximab were used. For each antibody, dose response curves were made at 10, 1, 0.1 and 0.01  $\mu$ g/ml (from left to right). Antibody 6 and reference antibodies are shown in panel (a); Isotype controls are shown in panel (b). Data is normalized for response to cetuximab (set to 100%). Experiment was performed as indicated in Experimental section 10.

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

The term "antibody" as used throughout the present specification refers to a monoclonal antibody (mAb) comprising two heavy chains and two light chains. Antibodies may be of any isotype such as IgA, IgE, IgG, or IgM antibodies. Antibodies may also be of an IgGA cross-isotype (Kelton *et al. Chemistry and Biology*, 2014, 21, 1603-1609). Preferably, the antibody is an IgG antibody, e.g. an IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> or IgG<sub>4</sub> antibody, more preferably an IgG<sub>1</sub> or IgG<sub>2</sub>

antibody. The term “immunoglobulin” (Ig) is used interchangeably with antibody herein. An antibody according to the invention preferably is a humanized or human antibody.

The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains (an IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called J chain, and therefore contain 10 antigen-binding sites, while secreted IgA antibodies can polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain units along with the J chain). In the case of IgGs, the 4-chain unit is generally about 150,000 Daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. However, IgGs may lack one or more of the disulfide bonds, while retaining their function. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (VH) followed by three constant domains (CH) for each of the  $\alpha$  and  $\gamma$  chains and four CH domains for  $\mu$  and  $\epsilon$  isotypes. Generally, the H chain comprises a hinge region, typically between the first and second constant region. Each L chain has at the N-terminus, a variable domain (VL) followed by a constant domain (CL) at its other end. The VL is aligned with the VH and the CL is aligned with the first constant domain of the heavy chain (CH1). Particular amino acid residues are believed to form an interface between the L chain and H chain variable domains. The pairing of a VH and VL together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, page 71 and Chapter 6 (1994).

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively. The  $\alpha$  and  $\gamma$  classes are further divided into subclasses on the basis of relatively minor differences in CH sequence and function, e.g., humans express the following subclasses: IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub>, and IgA<sub>2</sub>.

Preferably, an antibody of the invention is a humanized or human antibody. Even more preferably, the antibody is a humanized or human IgG antibody, most preferably a humanized or human IgG<sub>1</sub> mAb. The antibody may have  $\kappa$  or  $\lambda$  light chains, preferably  $\kappa$  light chains (for example as shown in SEQ ID NO:26), i.e., a humanized or human IgG<sub>1</sub>- $\kappa$  antibody. An antibody

of the invention may comprise a constant region that is engineered, for example one or more mutations may be introduced to e.g. increase half-life and/or decrease effector function.

The terms "monoclonal antibody" and "mAb" as used herein refer to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. There are several ways known in the art to generate monoclonal antibodies. For example, the monoclonal antibodies useful in the present invention may be generated by immunizing animals with a mixture of peptides representing the desired antigen. Subsequently, B-lymphocytes may be isolated and fused with myeloma cells or single B-lymphocytes may be 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, suitable B-cells or lymphoma may be lysed, 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 "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The variable domain mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the approximately 110-amino acid span of the variable domains. Instead, the variable regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" (HVRs) or "complementarity determining regions" (CDRs) that are each approximately 9-12 amino acids long, but may be shorter or longer, as for example HCDR1 in the mAbs of the present invention that has a length of 5 amino acid residues. The variable domains of heavy and light chains of naturally occurring antibodies each comprise four FRs, largely adopting a  $\beta$ -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al. Sequences of Proteins of Immunological*

Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in ADCC and/or ADCP. The variable domain of the heavy chain may be referred to as "VH". The variable domain of the  
5 light chain may be referred to as "VL".

The term "antigen-binding fragment" as used throughout the present specification includes Fab, Fab', F(ab')<sub>2</sub>, Fv, scFv and rIgG fragments as long as they exhibit the desired biological and/or immunological activity. The term "antigen-binding fragment" further includes a single chain (sc) antibody, a single domain (sd) antibody, a diabody, or a minibody.  
10 For example, papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (VH), and the first constant domain of one heavy chain (CH1). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site.  
15 Pepsin treatment of an antibody yields a single large F(ab')<sub>2</sub> fragment which roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having an additional few residues at the C-terminus of the CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine  
20 residue(s) of the constant domains bear a free thiol group. F(ab')<sub>2</sub> antibody fragments originally were produced as pairs of Fab' fragments, which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known. The Fc fragment comprises the C-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, which region is also the part  
25 recognized by Fc receptors (FcR) found on certain types of cells. "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy and one light chain variable region domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy and one light chain variable domain can be covalently linked by a flexible peptide linker such that the heavy and light chains  
30 can associate in a "dimeric" structure analogous to that in a two-chain Fv species. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although

at a lower affinity than the entire binding site. "Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994). "rIgG" refers to reduced IgG, approximately 75,000 Daltons, and may be produced by selectively reducing just the hinge region disulfide bonds, for example using a mild reducing agent such as 2-mercaptoethylamine (2-MEA).

The term "anti-SIRP $\alpha$  antibody" or "an antibody that binds to SIRP $\alpha$ " refers to an antibody that is capable of binding SIRP $\alpha$  with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting SIRP $\alpha$ . Preferably, the extent of binding of an anti-SIRP $\alpha$  antibody to an unrelated, non-SIRP protein is less than about 10% of the binding of the antibody to SIRP $\alpha$  as measured, e.g., by a radioimmunoassay (RIA), surface Plasmon resonance (SPR) or enzyme-linked immunosorbent assay (ELISA). Binding to non-related targets may also be profiled using a cell microarray technology, such as for example by Retrogenix<sup>TM</sup>.

An "isolated antibody" is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with therapeutic uses of the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated antibody will be prepared by at least one purification step.

"Binding affinity" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The binding affinity of a molecule X for its partner Y as used

throughout the present specification, refers to the equilibrium dissociation constant (also referred to as “binding constant”;  $K_D$ ) of a particular antigen-antibody interaction. The  $K_D$  is the ratio of the rate of dissociation ( $k_{off}$ ) to the association rate ( $k_{on}$ ). Consequently,  $K_D$  equals  $k_{off}/k_{on}$  and is expressed as a molar concentration (M). It follows that the smaller the  $K_D$ , the stronger the affinity of binding. Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative embodiments are described in the following. Typically,  $K_D$  values are determined by using surface plasmon resonance (SPR), typically using a biosensor system (e.g. BIAcore<sup>TM</sup>) using methods known in the art (e.g. E.S. Day *et al. Anal. Biochem.* 2013, 440, 96–107), such as for example described in the Example section. Alternatively, the term “binding affinity” may also refer to the concentration of antibody that gives half-maximal binding ( $EC_{50}$ ) determined with e.g. an ELISA assay or as determined by flow cytometry.

An antibody “which binds” an antigen of interest or antigens of interest, e.g. a SIRP $\alpha$  antigen target, is one that binds the antigen with sufficient affinity such that the antibody is useful as a therapeutic agent in targeting a cell or tissue expressing the antigen(s), and does not significantly cross-react with other proteins. For example, an anti-SIRP $\alpha$  antibody according to the present invention binds to human SIRP $\alpha$  – that is, at least to human SIRP $\alpha_1$  and human SIRP $\alpha_{BIT}$ , preferably to cynomolgus SIRP $\alpha$  – and possibly to SIRP $\beta_{1v1}$  and/or SIRP $\beta_{1v2}$ , whereas it does not bind to SIRP $\gamma$  or unrelated proteins. In such embodiments, the extent of binding of the antibody to a “non-target” protein will preferably be less than about 10%, more preferably less than about 5%, more preferably less than about 2%, more preferably less than about 1% of the binding of the antibody to its particular target protein as determined by fluorescence activated cell sorting (FACS) analysis or radioimmunoprecipitation assay (RIPA). With regard to the binding of an antibody to a target molecule, the term “specific binding” or “specifically binds to” or is “specific for” a particular polypeptide or an epitope on a particular polypeptide target means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target, for example, an excess of non-labelled target. In this case, specific binding is indicated if the binding of the labelled target to a probe is

competitively inhibited by excess unlabelled target. The term "specific binding" or "specifically binds to" or is "specific for" particular polypeptide(s) or an epitope on particular polypeptide target(s) as used herein can be exhibited, for example, by a molecule having a  $K_D$  for the target (which may be determined as described above) of at least about  $10^{-7}$  M, preferably at least about 5  $10^{-8}$  M, more preferably at least about  $10^{-9}$  M, even more preferably at least about  $10^{-10}$  M, even more preferably at least about  $10^{-11}$  M, even more preferably at least about  $10^{-12}$  M, or greater as determined by SPR at 25°C.

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 10 between an antibody and its antigen with an  $EC_{50}$  larger than 1500 ng/ml as determined using an ELISA assay, and/or where limited or no specific binding is observed between the immobilized antigen and the antibody as preferably determined by SPR at 25°C, such as for example when the  $K_D$  between an antibody and an antigen is higher than for example  $10^{-7}$  M, higher than  $10^{-6}$  M or even higher, as determined by SPR at 25°C.

15 The term "high affinity" as used herein refers to a binding affinity between an antibody and its antigen wherein the  $K_D$  is typically less than  $10^{-8}$  M,  $10^{-9}$  M,  $10^{-10}$  M,  $10^{-11}$  M, or even lower, as determined by SPR at 25°C, as described in the Examples.

"Humanized" forms of non-human (e.g., rodent) antibodies are antibodies (e.g., non-human-human chimeric antibodies) that contain minimal sequences derived from the non- 20 human antibody. Various methods for humanizing non-human antibodies are known in the art. For example, the antigen-binding CDRs in the variable regions (VRs) of the H chain (VH) and L chain (VL) are derived from antibodies from a non-human species, commonly mouse, rat or rabbit. These non-human CDRs are combined with human framework regions (FR1, FR2, FR3 and FR4) of the VH and VL regions, in such a way that the functional properties of the 25 antibodies, such as binding affinity and specificity, are at least partially retained. Selected amino acids in the human FRs may be exchanged for the corresponding original non-human species amino acids to further refine antibody performance, such as to improve binding affinity, while retaining low immunogenicity. Alternatively, non-human antibodies can be humanized by modifying their amino acid sequence to increase similarity to antibody variants produced 30 naturally in humans. For example, 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. Exemplary methods for humanization of non-human antibodies are the method of Winter and co-workers (Jones *et al.* *Nature* 1986, 321, 522-525;

Riechmann *et al. Nature* 1988, 332, 323-327; Verhoeven *et al. Science* 1988, 239, 1534-1536), by substituting CDRs for the corresponding sequences of a human antibody.

The thus humanized variable regions will be typically combined with human constant regions. In general, the humanized antibody will comprise typically two variable domains in which all or substantially all of the CDRs correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al. Nature* 1986, 321, 522-525; Riechmann *et al. Nature* 1988, 332, 323-327; and Presta. *Curr. Op. Struct. Biol.* 1992, 2, 593-596. See also the following review articles and references cited therein: Vaswani and Hamilton. *Ann. Allergy, Asthma and Immunol.* 1998, 1, 105-115; Harris. *Biochem. Soc. Transactions* 1995, 23, 1035-1038; and Hurle and Gross. *Curr. Op. Biotech.* (1994), 5, 428-433.

The term "hypervariable region", "HVR", when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops that are responsible for antigen binding. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of hypervariable region delineations are in use and are encompassed herein. The hypervariable regions generally comprise amino acid residues from a "complementarity determining region" or "CDR" (e.g., residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the VL, and around about 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the VH when numbered in accordance with the Kabat numbering system; Kabat *et al. Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991); and/or those residues from a "hypervariable loop" (e.g., residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the VL, and 26-32 (H1), 52-56 (H2) and 95-101 (H3) in the VH when numbered in accordance with the Chothia numbering system; Chothia and Lesk. *J. Mol. Biol.* 1987, 196, 901-917); and/or those residues from a "hypervariable loop"/CDR (e.g., residues 27-38 (L1), 56-65 (L2) and 105-120 (L3) in the VL, and 27-38 (H1), 56-65 (H2) and 105-120 (H3) in the VH when numbered in accordance with the IMGT numbering system; Lefranc *et al. Nucl. Acids Res.* 1999, 27, 209-212, Ruiz *et al. Nucl. Acids Res.* 2000, 28, 219-221). Optionally, the antibody has symmetrical insertions at one or more of the following points 28, 36 (L1), 63, 74-75 (L2) and 123 (L3) in the VL, and 28, 36 (H1), 63, 74-75 (H2) and 123 (H3) in the VH when numbered in accordance with Honneger and Plunkthun (*Mol. Biol.* 2001, 309, 657-670). Dondelinger *et al.* reviewed several of the numbering systems and their uses (Dondelinger *et*

*al. Frontiers in Immunology*, 2018, 9, Art 2278). The HVR/CDRs of the antibodies and antigen-binding fragments of the invention are preferably defined and numbered in accordance with the Kabat numbering system.

"Framework" or "FR" residues are those variable domain residues other than the  
5 hypervariable region residues herein defined.

A "blocking" antibody or an "antagonist(ic)" antibody as used herein means an antibody which partially or fully prevents the natural ligand to bind. For example, an anti-SIRP $\alpha$  blocking antibody prevents CD47 to bind to SIRP $\alpha$ . Preferred blocking antibodies or antagonist(ic) antibodies substantially or completely inhibit the biological activity of the antigen.

10 The term "epitope" is the portion of a molecule that is bound by an antigen-binding protein, e.g. an antibody. The term includes any determinant capable of specifically binding to an antigen-binding protein, such as an antibody or to a T-cell receptor. An epitope can be contiguous or non-contiguous (e.g., in a polypeptide, amino acid residues that are not  
15 contiguous to one another in the polypeptide sequence but that within the context of the molecule – i.e. the tertiary structure – are bound by the antigen-binding protein). In certain embodiments, epitopes may be mimetic in that they comprise a three dimensional structure that is similar to an epitope used to generate the antigen-binding protein, yet comprise none or only some of the amino acid residues found in that epitope used to generate the antigen-binding protein. Most often, epitopes reside on proteins, but in some instances may reside on other kinds  
20 of molecules, such as nucleic acids. Epitope determinants may include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, sulfonyl or sulfate groups, and may have specific three dimensional structural characteristics, and/or specific charge characteristics. Generally, antibodies specific for a particular target antigen will preferentially recognize an epitope on the target antigen in a complex mixture of proteins and/or  
25 macromolecules. The extracellular domain of SIRP $\alpha$  may for example harbour epitopes in domain d1, d2 or d3.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including wild-type sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG  
30 heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the C-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may

comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue.

5 A "functional Fc region" possesses an "effector function" of a wild-type sequence Fc region. Antibody effector functions vary with antibody isotype. Exemplary "effector functions" include complement component 1q (C1q) binding; CDC; Fc receptor binding; ADCC; ADCP; ADAP; down regulation of cell surface receptors (e.g. B-cell receptor; BCR), and B-cell activation. Such effector functions generally require the Fc region to be combined with a binding domain (e.g. an antibody variable domain) and can be assessed using various assays as  
10 disclosed, for example, in definitions herein.

A "wild-type sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Wild-type sequence human Fc regions include a wild-type sequence human IgG<sub>1</sub> Fc region (non-A and A allotypes); a wild-type sequence human IgG<sub>2</sub> Fc region; a wild-type sequence human IgG<sub>3</sub> Fc region; and a wild-type  
15 sequence human IgG<sub>4</sub> Fc region as well as naturally occurring variants thereof.

A "variant Fc region" comprises an amino acid sequence which differs from that of a wild-type sequence Fc region by virtue of at least one amino acid modification, preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid substitution compared to a wild-type sequence Fc region or to the Fc region of a parent  
20 polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a wild-type sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a wild-type sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at  
25 least about 95% homology therewith.

"Antibody-dependent cell-mediated cytotoxicity" (also referred to as "antibody-dependent cellular cytotoxicity") or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., Natural Killer (NK) cells, neutrophils, and monocytes/macrophages) enable these cytotoxic effector cells to bind  
30 specifically to an antigen-bearing target cell. The NK cells express FcγRIIIA only, whereas monocytes express FcγRI, FcγRIIA/B and FcγRIIIA. Neutrophils, which are the most abundant leukocytes in human blood, also mediate ADCC and this generally depends on FcγRIIA (Zhao *et al. Natl Acad Sci U S A.* 2011, 108(45), 18342-7; Treffers *et al. Eur J Immunol.* 2018, 48(2), 344-354; Matlung *et al. Cell Rep.* 2018, 23(13), 3946-3959). FcR expression on haematopoietic

cells is summarized in Table 2 on page 33 of Bruhns and Jönsson *Immunol Rev.* 2015, 268(1), 25-51. To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 or such as described in the Examples may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC), containing a mixture of monocytes and NK cells, or isolated monocytes, neutrophils or NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes *et al. Proc. Natl. Acad. Sci. (USA)* 1998, 95, 652-656 or in well-known tumour models such as the B16F10 model described in Zhao *et al.* PNAS 2011, see above. "Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a wild-type sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (see review M. in Daëron. *Annu. Rev. Immunol.* 1997, 15, 203-234). FcRs are reviewed in Ravetch and Kinet. *Annu. Rev. Immunol.* 1991, 9, 457-492; Capel *et al. Immunomethods* 1994, 4, 25-34; and de Haas *et al. J. Lab. Clin. Med.* 1995, 126, 330-341. Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express FcγRIIA (neutrophils or monocytes) or FcγRIIIA (NK cells or monocytes) and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include PBMCs, NK cells, monocytes, macrophages, and neutrophils, with neutrophils being preferred. The effector cells may be isolated from a native source, e.g., from blood, preferably from human blood.

The term "therapeutic antibody" as used herein 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 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. In the present specification the term “therapeutic antibody” does not include an anti-SIRP $\alpha$  antibody. The term “therapeutic antibody” as used herein means an anti-cancer antibody such as for example an anti-CD20, anti-HER2, anti-GD2, anti-EGFR or anti-CD70 antibody.

5       “Sequence identity” and “sequence similarity” can be determined by alignment of two amino acid sequences or two nucleotide sequences using global or local alignment algorithms, depending on the length of the two sequences. Sequences of similar lengths are preferably aligned using global alignment algorithms (e.g. Needleman and Wunsch) which aligns the sequences optimally over the entire length, while sequences of substantially different lengths  
10 are preferably aligned using a local alignment algorithm (e.g. Smith Waterman). Sequences may then be referred to as “substantially identical” or “essentially similar” when they (when optimally aligned by for example the programs GAP or BESTFIT using default parameters) share at least a certain minimal percentage of sequence identity (as defined below). GAP uses the Needleman and Wunsch global alignment algorithm to align two sequences over their entire  
15 length (full length), maximizing the number of matches and minimizing the number of gaps. A global alignment is suitably used to determine sequence identity when the two sequences have similar lengths. Generally, the GAP default parameters are used, with a gap creation penalty = 50 (nucleotides) / 8 (proteins) and gap extension penalty = 3 (nucleotides) / 2 (proteins). For nucleotides the default scoring matrix used is nwsgapdna and for proteins the default scoring  
20 matrix is Blosom62 (Henikoff & Henikoff, PNAS 1992, 89, 915-919). Sequence alignments and scores for percentage sequence identity may be determined using computer programs, such as the GCG Wisconsin Package, Version 10.3, available from Accelrys Inc., 9685 Scranton Road, San Diego, CA 92121-3752 USA, or using open source software, such as the program “needle” (using the global Needleman Wunsch algorithm) or “water” (using the local Smith  
25 Waterman algorithm) in EmbossWIN version 2.10.0, using the same parameters as for GAP above, or using the default settings (both for ‘needle’ and for ‘water’ and both for protein and for DNA alignments, the default Gap opening penalty is 10.0 and the default gap extension penalty is 0.5; default scoring matrices are Blossum62 for proteins and DNABFull for DNA). When sequences have substantially different overall lengths, local alignments, such as those  
30 using the Smith Waterman algorithm, are preferred. Alternatively, percentage similarity or identity may be determined by searching against public databases, using algorithms such as FASTA, BLAST, etc.

Once two amino acid sequences are aligned using any of the above alignment programs, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a

given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y, where X is the number of amino acid residues scored as identical matches by the sequence alignment  
5 program in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

Optionally, in determining the degree of amino acid similarity, so-called "conservative  
10 amino acid substitutions" may be taken into account. "Conservative amino acid substitutions" refer to the interchangeability of residues having similar side chains. For example, a group of amino acid residues with aliphatic side chains is glycine, alanine, valine, leucine and isoleucine; a group of a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a  
15 group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Exemplary conservative  
20 substitutions for each of the naturally occurring amino acids are as follows: ala to ser; arg to lys; asn to gln or his; asp to glu; cys to ser or ala; gln to asn; glu to asp; gly to pro; his to asn or gln; ile to leu or val; leu to ile or val; lys to arg, gln or glu; met to leu or ile; phe to met, leu or tyr; ser to thr; thr to ser; trp to tyr; tyr to trp or phe; and val to ile or leu.

A "nucleic acid construct" or "nucleic acid vector" is herein understood to mean a man-  
25 made nucleic acid molecule resulting from the use of recombinant DNA technology. The term "nucleic acid construct" therefore does not include naturally occurring nucleic acid molecules although a nucleic acid construct may comprise (parts of) naturally occurring nucleic acid molecules. The terms "expression vector" or "expression construct" refer to nucleotide sequences that are capable of effecting expression of a gene in host cells or host organisms  
30 compatible with such sequences. These expression vectors typically include at least suitable transcription regulatory sequences and, optionally, 3' transcription termination signals. Additional factors necessary or helpful in effecting expression may also be present, such as expression enhancer elements. The expression vector will be introduced into a suitable host cell and be able to effect expression of the coding sequence in an *in vitro* cell culture of the host

cell. The expression vector will be suitable for replication in the host cell or organism of the invention.

As used herein, the term "promoter" or "transcription regulatory sequence" refers to a nucleic acid fragment that functions to control the transcription of one or more coding sequences, and is located upstream with respect to the direction of transcription of the transcription initiation site of the coding sequence, and is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites and any other DNA sequences, including, but not limited to transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art to act directly or indirectly to regulate the amount of transcription from the promoter. A "constitutive" promoter is a promoter that is active in most tissues under most physiological and developmental conditions. An "inducible" promoter is a promoter that is physiologically or developmentally regulated, e.g. by the application of a chemical inducer.

As used herein, the term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a transcription regulatory sequence is operably linked to a coding sequence if it affects the transcription of the coding sequence. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two protein encoding regions, contiguous and in reading frame.

The term "ADC" as used herein refers to a cytotoxic drug conjugated to a therapeutic antibody or an antigen-binding fragment thereof as defined hereinabove via a linker. The antibody or antigen-binding fragment thereof in the ADC is not an anti-SIRP $\alpha$  antibody. Typically, the cytotoxic drugs are highly potent, e.g. a duocarmycin, calicheamicin, pyrrolobenzodiazepine (PBD) dimer, maytansinoid or auristatin derivative. The linker may be cleavable, e.g. comprising the cleavable dipeptide valine-citrulline (vc) or valine-alanine (va), or non-cleavable, e.g. succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC).

### Antibodies of the invention or fragments thereof

No approved therapeutics directed against SIRP $\alpha$  are currently available, although SIRP $\alpha$  has been shown to play an important role in tumour immune evasion mechanisms.

SIRP $\alpha$  (also known as CD172 antigen-like family member A, CD172a, SHPS-1, MyD-1) is a member of the family of signal regulatory proteins (SIRP), transmembrane glycoproteins

with extracellular Ig-like domains present on immune effector cells. CD47 (also known as integrin-associated protein, IAP) is a ligand of SIRP $\alpha$ . 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.

5 SIRP $\alpha_{BIT}$  (v1) and SIRP $\alpha_1$  (v2) are the two most common and most divergent (13 residues different) polymorphs (Hatherley *et al. J. Biol. Chem.* 2014, 289(14), 10024-10028). Besides by CD47, SIRP $\alpha$  can also be bound by surfactant proteins (SP-A and SP-D; Gardai *et al* 2003, Janssen *et al.* 2008, Fournier *et al.* 2012). In fact, CD47 can bind to SIRP $\alpha$  domain D1 whereas SP-D can (simultaneously) bind to SIRP $\alpha$  domain D3. Like SIRP $\alpha$ , the surfactant proteins are

10 implicated in innate immune/inflammatory responses towards e.g. apoptotic cells and microorganisms, involving macrophages and/or neutrophils. Other biochemically characterized human SIRP family members are SIRP $\beta_1$ , and SIRP $\gamma$ . SIRP-family nomenclature is described in van den Berg *et al. J Immunology* 2005, 175(12), 7788-9.

SIRP $\beta_1$  (also known as CD172B) does not bind CD47 (van Beek *et al. J. Immunol.* 2005, 175(12), 7781-7787, 7788-7789) and at least two SIRP $\beta_1$  polymorphic variants are known, SIRP $\beta_{1v1}$  (ENSP00000371018) and SIRP $\beta_{1v2}$  (ENSP00000279477). Although the natural ligand of SIRP $\beta_1$  is yet unknown, *in vitro* studies using anti-SIRP $\beta_1$  specific antibodies show that engagement of SIRP $\beta_1$  promotes phagocytosis in macrophages by inducing the tyrosine phosphorylation of DAP12, Syk, and SLP-76, and the subsequent activation of a MEK-MAPK-

20 myosin light chain kinase cascade (Matozaki *et al. J. Biol. Chem.* 2004, 279(28), 29450-29460). Human SIRP $\beta_1$  is expressed in myeloid cells, such as monocytes and granulocytes, but not in lymphocytes (Hayashi *et al. J. of Biol Chem.* 2004, 279(28); Dietrich *et al. The Journal of Immunology* 2000, 164, 9-12).

SIRP $\gamma$  (also known as CD172G) is expressed on T-cells and activated NK-cells and binds

25 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 (Stefanidakis *et al. Blood* 2008, 112, 1280-1289). WO2017/178653 discloses that due to the

30 high similarity of sequences between SIRP $\alpha$  and SIRP $\gamma$ , in particular in the region that interacts with CD47, the anti-SIRP $\alpha$  antibodies of the prior art also bind SIRP $\gamma$  and have undesirable effects in humans such as inhibition of the proliferation of T-cells and a decrease of the immune response. Thus, an anti-SIRP $\alpha$  antibody of the invention, which does not bind to SIRP $\gamma$  on T-cells, is hypothesized to have less or no inhibition of T-cell extravasation and/or less or no

inhibition of transendothelial migration of T-cells as opposed to an anti-SIRP $\alpha$  antibody that does bind SIRP $\gamma$  on T-cells.

The present invention relates to antagonistic anti-SIRP $\alpha$  antibodies which exhibit specific binding to at least the two predominant human SIRP $\alpha$  polymorphic variants SIRP $\alpha_{BIT}$  and SIRP $\alpha_1$ . Preferably, the anti-SIRP $\alpha$  antibodies of the invention have reduced, low or most preferable no affinity to human SIRP $\gamma$  (preferably as measured by CD3<sup>+</sup> T-cell FACS staining or surface plasmon resonance (preferably BiaCore<sup>TM</sup>) according to the Example section). Preferably, the anti-SIRP $\alpha$  antibodies of the invention have reduced or low affinity to human SIRP $\beta_{1v1}$  and/or human SIRP $\beta_{1v2}$ . Preferably, they increase the ADCC and/or ADCP of a therapeutic antibody. In a preferred embodiment, an anti-SIRP $\alpha$  antibody according to the invention also binds to cynomolgus monkey SIRP $\alpha$ .

Antagonistic antibodies have affinity for a specific antigen, and binding of the antibody to its antigen inhibits the function of an agonist or inverse agonist at receptors. In the present case, it is hypothesized that binding of an antagonistic anti-SIRP $\alpha$  antibody to SIRP $\alpha$  will prevent binding of CD47 to SIRP $\alpha$ . Antagonistic anti-SIRP $\alpha$  antibodies may bind to the same site where CD47 binds, i.e. an orthosteric site, preventing ligation of SIRP $\alpha$  by CD47 and consequently inhibiting the signalling that negatively regulates the Fc-receptor-dependent action of immune effector cells. Alternatively, antagonistic anti-SIRP $\alpha$  antibodies may bind in close vicinity to the site where CD47 binds, preventing interaction between CD47 and SIRP $\alpha$  through steric hindrance. Alternatively, an antibody distal to the CD47 interaction site may block CD47/SIRP $\alpha$  binding, for example by inducing a non-receptive conformation. Without wishing to be bound by any theory, it is hypothesized that blocking CD47-binding to SIRP $\alpha$  reduces or prevents the SIRP $\alpha$  signalling cascade, preferably by at least 60%, more preferably by at least 65%, at least 70%, at least 75%, most preferably by at least 80% when measured as indicated in the Examples at an anti-SIRP $\alpha$  antibody concentration of 3.3  $\mu$ g/ml.

If an antibody binds also to other antigens than the target, such as for example to SIRP $\beta_{1v1}$ , SIRP $\beta_{1v2}$  and SIRP $\gamma$ , especially when the antibody is bound to those antigens with high affinity, such antigens may function as an antibody sink. Therefore, it is hypothesized that by minimizing off-target antibody binding, any antibody sink effect is reduced, which may result in efficacy at a lower dose or a higher effect at the same dose as compared to an antibody which further has the same characteristics.

The role of SIRP $\beta_1$  is at present poorly understood. Therefore, it is preferred that an antibody of the present invention has relatively low binding affinities to both of the polymorphic variants (SIRP $\beta_{1v1}$  and SIRP $\beta_{1v2}$ ). However, it has been reported that activation of SIRP $\alpha$

(signalling via Immunoreceptor Tyrosine-based Inhibitory Motifs, ITIMs) and SIRP $\beta_1$  (signalling via Immunoreceptor Tyrosine-based Activating Motifs, ITAMs) have opposing effects on the function of immunologic effector cells such as macrophages and PMNs/neutrophils (van Beek *et al. J. Immunol.* 2005, 175(12), 7781-7787, 7788-7789).

5 Furthermore, it has been reported that triggering of the murine SIRP $\beta_1$  receptor promotes phagocytosis in macrophages (Hayashi *et al. J. Biol. Chem.* 2004, 279(28), 29450-29460). Liu *et al. (J. Biol. Chem.* 2005, 280 (43), 36132-36140) state that antibody-mediated ligation of SIRP $\beta_1$  enhanced fMLP-driven PMN transepithelial migration. Without wishing to be bound by any theory, it is conceivable that anti-SIRP $\alpha$  blocking antibodies that also bind to SIRP $\beta_1$

10 may have opposite effects on SIRP $\alpha$  and SIRP $\beta_1$ . In order to obtain maximal stimulation of an anti-tumour cell response, there is a need for antibodies that stimulate the activity of innate immune cells (e.g. PMNs/neutrophils and/or macrophages) by blocking SIRP $\alpha$ , while limiting or avoiding affecting SIRP $\beta_1$  functioning, and simultaneously avoiding blocking adaptive immune cells, i.e. T-cells via binding to SIRP $\gamma$ .

15 The antibodies of the present invention preferably have all of the following characteristics: (i) they are able to block CD47 binding to SIRP $\alpha_{BIT}$  and SIRP $\alpha_1$ , (ii) they do not bind to human CD3<sup>+</sup> T-cells, and (iii) they are able to reduce SIRP $\alpha$  signalling. Preferably, they have reduced or low binding to SIRP $\beta_{1V1}$  and/or SIRP $\beta_{1V2}$  as measured by SPR. In contrast, known anti-SIRP $\alpha$  antibodies do not combine all desired specificities in a single antibody: all

20 of the antibodies that are able to block CD47 binding to SIRP $\alpha_{BIT}$  and SIRP $\alpha_1$  also show binding to SIRP $\gamma$ , whereas HEFLB is able to block SIRP $\alpha_{BIT}$  without binding to SIRP $\gamma$ , but does not have the ability to bind, let alone to block, SIRP $\alpha_1$ . Some other prior art antibodies that are able to bind both SIRP $\alpha_1$  and SIRP $\alpha_{BIT}$  without binding SIRP $\gamma$  are not able to block CD47 binding to SIRP $\alpha$  and hence do not, or only at a high IC<sub>50</sub> concentration, block SIRP $\alpha$  signalling as

25 measured by blocking of the recruitment of SHP-1. Despite being able to bind and block both SIRP $\alpha_1$  and SIRP $\alpha_{BIT}$ , SE5A5 is not capable of blocking SIRP $\alpha$  signalling as measured by blocking of the recruitment of SHP-1. These characteristics are determined as described or referred to in the Examples.

In a first aspect, the invention provides an antibody or an antigen-binding fragment thereof that binds to SIRP $\alpha$ . An anti-SIRP $\alpha$  antibody of the invention preferably is an isolated antibody. Preferably, an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to the invention binds to a primate SIRP $\alpha$ , more preferably to a human SIRP $\alpha$ , most preferably at least to the allelic variants SIRP $\alpha_1$  and SIRP $\alpha_{BIT}$ . In a preferred embodiment, the antibody or antigen-binding fragment thereof has reduced binding as compared to prior art antibodies, more

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preferably has low binding, most preferably does not bind to SIRP $\gamma$ , preferably as measured using human CD3<sup>+</sup> T-cell FACS staining according to the Examples or as Biacore experiment as shown in the Examples. Preferably, the antibody or antigen-binding fragment thereof increases the ADCC and/or ADCP of therapeutic antibodies. Preferably, an anti-SIRP $\alpha$  antibody of the invention increases the ADCC of 10  $\mu$ g/ml of trastuzumab at 0.1  $\mu$ g/ml of anti-SIRP $\alpha$  antibody at least 1.2; more preferably at least 1.3; more preferably at least 1.4; more preferably at least 1.5; more preferably at least 1.6; more preferably at least 1.7; more preferably at least 1.8; more preferably at least 1.9; more preferably at least 2.0; more preferably at least 2.1; more preferably at least 2.2; most preferably at least 2.3-fold as compared to trastuzumab alone, when measured using the DELFIA or <sup>51</sup>Cr-release assay as used in the Examples.

Alternatively or in combination with any one of the other embodiments herein, in a preferred embodiment, an anti-SIRP $\alpha$  antibody of the invention is a humanized or human anti-SIRP $\alpha$  antibody and an antigen-binding fragment according to the invention is an antigen-binding fragment as defined hereinabove from a humanized or human anti-SIRP $\alpha$  antibody. More preferably, an anti-SIRP $\alpha$  antibody of the invention is a humanized anti-SIRP $\alpha$  antibody and an antigen-binding fragment of the invention is derived from a humanized anti-SIRP $\alpha$  antibody.

A humanized antibody according to the invention or an antigen-binding fragment thereof preferably elicits little to no immunogenic response against the antibody in a subject to which the antibody or fragment is administered. The original non-human antibody, for example a rodent antibody such as a mouse or a rat antibody, or a rabbit antibody or the non-human - human chimeric antibody may potentially elicit a human anti-non-human animal antibody response, in which case the humanized antibody according to the invention or antigen-binding fragment thereof elicits and/or is expected to elicit an immunogenic response at a substantially reduced level compared to the original non-human antibody or compared to the non-human - human chimeric antibody in a host subject. Preferably, the humanized antibody elicits and/or is expected to elicit a minimal or no human anti-non-human antibody response. Immunogenicity testing can be performed as known by methods in the art, such as described in Baker *et al. Immunogenicity of protein therapeutics* 2010, 1(4), 314-322; Harding *et al. MAbs* 2010, 2(3), 256-265; or Joubert *et al. PLoS.One* 2016, 11(8), e0159328. Most preferably, an antibody of the invention elicits a human anti-non-human animal antibody response, particularly a human anti-rabbit antibody (HARA) response, that is at or less than a clinically-acceptable level. It is further important that antibodies be humanized with retention of high affinity for the antigen and other favourable biological properties.

The CDRs may be determined using the approach of Kabat (in Kabat *et al.* Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD 1991, NIH publication no. 91-3242, pp. 662, 680, 689), Chothia (Chothia and Lesk. *J. Mol. Biol.* 1987, 196, 901-917; Antibody Engineering Vol. 2, Chapter 3 by Martin, 5 2010, Kontermann and Dübel Eds. Springer-Verlag Berlin Heidelberg) or IMGT (Lefranc. *The Immunologist* 1999, 7, 132-136). The CDRs of the heavy chain and the light chain as used herein are determined using Kabat and shown in the sequence listing under the SEQ ID NO as indicated in Table 1a. 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 10 expression "Eu numbering" refers to the Eu index as in Kabat *et al.* Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD 1991, NIH publication no. 91-3242, pp. 662, 680, 689.

In a preferred embodiment, the present invention relates to an anti-SIRP $\alpha$  antibody, preferably a humanized anti-SIRP $\alpha$  antibody, or an antigen-binding fragment thereof, 15 comprising heavy chain complementarity determining regions (HCDR) and light chain complementarity determining regions (LCDR) HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, selected from the group consisting of: (a) HCDR1 comprises SHGIS (SEQ ID NO:36); HCDR2 comprises TIGTGVITYFASWAKG (SEQ ID NO:44); HCDR3 comprises GSAWNDPFDP (SEQ ID NO:45); LCDR1 comprises QASQSVYGNNDLA (SEQ ID 20 NO:39); LCDR2 comprises LASTLAT (SEQ ID NO:40); LCDR3 comprises LGGGDDEADNT (SEQ ID NO:41); (b) HCDR1 comprises SYVMG (SEQ ID NO:30); HCDR2 comprises IISSSGSPYYASWVNG (SEQ ID NO:31); HCDR3 comprises VGPLGVDYFNI (SEQ ID NO:32); LCDR1 comprises RASQSINSYLA (SEQ ID NO:33); LCDR2 comprises SASFLYS (SEQ ID NO:34); LCDR3 comprises QSWHYISRSYT (SEQ ID 25 NO:35); (c) HCDR1 comprises SHGIS (SEQ ID NO:36); HCDR2 comprises TIGTGVITYYASWAKG (SEQ ID NO:37); HCDR3 comprises GSAWNDPFDY (SEQ ID NO:38); LCDR1 comprises QASQSVYGNNDLA (SEQ ID NO:39); LCDR2 comprises LASTLAT (SEQ ID NO:40); LCDR3 comprises LGGGDDEADNV (SEQ ID NO:46); (d) HCDR1 comprises SHGIS (SEQ ID NO:36); HCDR2 comprises TIGTGVITYYASWAKG 30 (SEQ ID NO:37); HCDR3 comprises GSAWNDPFDY (SEQ ID NO:38); LCDR1 comprises QASQSVYGNNDLA (SEQ ID NO:39); LCDR2 comprises LASTLAT (SEQ ID NO:40); LCDR3 comprises LGGGDDEADNT (SEQ ID NO:41); and (e) HCDR1 comprises SHGIS (SEQ ID NO:36); HCDR2 comprises TIGTGGITYYASWAKG (SEQ ID NO:42); HCDR3 comprises GSAWNDPFDI (SEQ ID NO:43); LCDR1 comprises QASQSVYGNNDLA (SEQ

ID NO:39); LCDR2 comprises LASTLAT (SEQ ID NO:40); LCDR3 comprises LGGGDDEADNT (SEQ ID NO:41). More preferably, an anti-SIRP $\alpha$  antibody according to the invention or an antigen-binding fragment thereof comprises HCDR and LCDR selected from the group consisting of: (a) HCDR1 comprises SHGIS (SEQ ID NO:36); HCDR2  
 5 comprises TIGTGVITYFASWAKG (SEQ ID NO:44); HCDR3 comprises GSAWNDPFDLP (SEQ ID NO:45); LCDR1 comprises QASQSVYGNNDLA (SEQ ID NO:39); LCDR2 comprises LASTLAT (SEQ ID NO:40); LCDR3 comprises LGGGDDEADNT (SEQ ID NO:41); and (b) HCDR1 comprises SYVMG (SEQ ID NO:30); HCDR2 comprises IISSSGSPYYASWVNG (SEQ ID NO:31); HCDR3 comprises VGPLGVDYFNI (SEQ ID  
 10 NO:32); LCDR1 comprises RASQSINSYLA (SEQ ID NO:33); LCDR2 comprises SASFLYS (SEQ ID NO:34); LCDR3 comprises QSWHYISRSYT (SEQ ID NO:35).

Alternatively or in combination with any one of the preceding embodiments, in a preferred embodiment, an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to the invention, has one or more of the following functional properties.

15 Preferably, the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human SIRP $\alpha_1$  with a  $K_D$  below  $10^{-9}$  M, more preferably with a  $K_D$  below  $10^{-10}$  M, even more preferably with a  $K_D$  below  $10^{-11}$  M, as analysed by surface plasmon resonance (preferably BiaCore<sup>TM</sup>) at 25°C using human SIRP $\alpha_1$  extracellular domain as shown in SEQ ID NO:51. Preferably, the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human  
 20 SIRP $\alpha_{BIT}$  with a  $K_D$  below  $10^{-9}$  M, more preferably with a  $K_D$  below  $10^{-10}$  M, even more preferably with a  $K_D$  below  $10^{-11}$  M as analysed by surface plasmon resonance (preferably BiaCore<sup>TM</sup>) at 25°C using human SIRP $\alpha_{BIT}$  extracellular domain as shown in SEQ ID NO:52. Preferably, the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds cynomolgus monkey SIRP $\alpha$  with a  $K_D$  below  $10^{-7}$  M, more preferably with a  $K_D$  below  $10^{-8}$  M, even more  
 25 preferably with a  $K_D$  below  $10^{-9}$  M as analysed by surface plasmon resonance (preferably BiaCore<sup>TM</sup>) at 25°C using cynomolgus SIRP $\alpha$  extracellular domain as shown in SEQ ID NO:56. Preferably, the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof does not detectably bind human SIRP $\gamma$  as measured by CD3<sup>+</sup> T-cell FACS staining according to the Examples. Preferably, the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof does not bind  
 30 human SIRP $\gamma$  as analysed by surface plasmon resonance (preferably BiaCore<sup>TM</sup>) at 25°C using human SIRP $\gamma$  extracellular domain as shown in SEQ ID NO:55. Preferably, the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof is not immunogenic as determined by IL-2 ELISpot and/or T-cell proliferation assay. Preferably, the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human SIRP $\beta_{1v2}$  with moderate to low affinity as analysed by

surface plasmon resonance (preferably BiaCore™) at 25°C using human SIRPβ<sub>1v2</sub> extracellular domain as shown in SEQ ID NO:54. Preferably, the K<sub>D</sub> is above 10<sup>-10</sup> M, more preferably above 10<sup>-9</sup> M. Preferably, in combination with the moderate to low affinity binding of SIRPβ<sub>1v2</sub>, the anti-SIRPα antibody or the antigen-binding fragment thereof binds SIRPβ<sub>1v1</sub> with a K<sub>D</sub> above  
 5 10<sup>-11</sup> M, more preferably above 3x10<sup>-11</sup> M, even more preferably above 10<sup>-10</sup> M, even more preferably above 10<sup>-9</sup> M as analysed by surface plasmon resonance (preferably BiaCore™) at 25°C using human SIRPβ<sub>1v1</sub> extracellular domain as shown in SEQ ID NO:53. These assays are described or referred to in the Examples.

Alternatively or in combination with any one of the preceding embodiments, in a preferred embodiment, the invention relates to an anti-SIRPα antibody or an antigen-binding fragment thereof as defined hereinabove, wherein the antibody shows specific binding to both  
 10 human SIRPα<sub>BIT</sub> and human SIRPα<sub>1</sub> and does not detectably bind to human SIRPγ using CD3<sup>+</sup> T-cell staining and/or SPR, both as described in the Examples. In an embodiment, the anti-SIRPα antibody or an antigen-binding fragment thereof binds human SIRPα<sub>BIT</sub> with a K<sub>D</sub> below  
 15 10<sup>-9</sup> M, more preferably with a K<sub>D</sub> below 10<sup>-10</sup> M, even more preferably with a K<sub>D</sub> below 10<sup>-11</sup> M; and binds human SIRPα<sub>1</sub> with a K<sub>D</sub> below 10<sup>-8</sup> M, more preferably with a K<sub>D</sub> below 10<sup>-9</sup> M, more preferably with a K<sub>D</sub> below 10<sup>-10</sup> M, even more preferably with a K<sub>D</sub> below 10<sup>-11</sup> M, wherein the K<sub>D</sub> is measured with SPR at 25°C (see Examples). Preferably, the anti-SIRPα antibody of the invention or an antigen-binding fragment thereof binds to SIRPγ with a K<sub>D</sub>  
 20 higher than 10<sup>-8</sup> M, more preferably with a K<sub>D</sub> higher than 10<sup>-7</sup> M, more preferably with a K<sub>D</sub> higher than 10<sup>-6</sup> M, even more preferably with a K<sub>D</sub> higher than 10<sup>-5</sup> M, most preferably, where no binding can be detected, wherein the K<sub>D</sub> is measured with SPR at 25°C (see Examples).

Alternatively or in combination with any one of the preceding embodiments, in a preferred embodiment, an anti-SIRPα antibody or an antigen-binding fragment thereof according to the invention: (a) binds human SIRPα<sub>1</sub> with a K<sub>D</sub> below 10<sup>-8</sup> M, more preferably  
 25 with a K<sub>D</sub> below 10<sup>-9</sup> M, more preferably with a K<sub>D</sub> below 10<sup>-10</sup> M, even more preferably with a K<sub>D</sub> below 10<sup>-11</sup> M, as analysed by surface plasmon resonance (SPR; preferably by BiaCore™) at 25°C using human SIRPα<sub>1</sub> extracellular domain as shown in SEQ ID NO:51 (see Examples); (b) binds human SIRPα<sub>BIT</sub> with a K<sub>D</sub> below 10<sup>-9</sup> M, more preferably with a K<sub>D</sub> below 10<sup>-10</sup> M,  
 30 even more preferably with a K<sub>D</sub> below 10<sup>-11</sup> M as analysed by SPR (preferably by BiaCore™) at 25°C using human SIRPα<sub>BIT</sub> extracellular domain as shown in SEQ ID NO:52 (see Examples); (c) blocks CD47 binding to SIRPα<sub>1</sub> and SIRPα<sub>BIT</sub>, preferably as analysed by dissociation from captured CD47 by SPR (preferably by BiaCore™), more preferably as described in Example 6; and (d) does not detectably bind human SIRPγ as measured by CD3<sup>+</sup>

T-cell flow cytometry, preferably fluorescence-activated cell sorting (FACS) staining, and/or SPR, both as described in the Examples. Preferably, in combination with the previous embodiment, the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human SIRP $\beta_{1v2}$  with moderate to low affinity as analysed by SPR (preferably by BiaCore™) at 25°C using human SIRP $\beta_{1v2}$  extracellular domain as shown in SEQ ID NO:54. Preferably, the  $K_D$  is above  $10^{-10}$  M, more preferably above  $10^{-9}$  M. Preferably, in combination with the moderate to low affinity binding of SIRP $\beta_{1v2}$ , the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds SIRP $\beta_{1v1}$  with a  $K_D$  above  $10^{-11}$  M, more preferably above  $3 \times 10^{-11}$  M, even more preferably above  $10^{-10}$  M, even more preferably above  $10^{-9}$  M as analysed by SPR (preferably by BiaCore™) at 25°C using human SIRP $\beta_{1v1}$  extracellular domain as shown in SEQ ID NO:53. These assays are described or referred to in the Examples.

Alternatively or in combination with any one of the preceding embodiments, in a preferred embodiment the invention relates to an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to the invention, wherein: (a) the heavy chain variable domain of the antibody comprises 4 heavy chain framework regions, HFR1 to HFR4, and 3 complementarity determining regions HCDR1 to HCDR3 that are operably linked in the order HFR1-HCDR1-HFR2-HCDR2-HFR3-HCDR3-HFR4, wherein each of the heavy chain framework regions has at least 90%, at least 91%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99% or preferably has 100% amino acid identity with the framework amino acid sequence of any one of SEQ ID NOs 1, 3 – 6, 8, 10 – 15, or wherein HFR1 to HFR4 differ from any one of SEQ ID NOs 1, 3 – 6, 8, 10 – 15 in one or more of the amino acid substitutions as defined in Tables 8 to 11; and (b) the light chain variable domain of the antibody comprises 4 light chain framework regions, LFR1 to LFR4, and 3 complementarity determining regions LCDR1 to LCDR3 that are operably linked in the order LFR1-LCDR1-LFR2-LCDR2-LFR3-LCDR3-LFR4, wherein each of the light chain framework regions has at least 90%, at least 91%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99% or preferably has 100% amino acid identity with the framework amino acid sequence of any one of SEQ ID NOs 2, 7 or 9, or wherein LFR1, LFR2 and/or LFR4 differ from any one of SEQ ID NOs 2, 7 or 9 in one or more amino acid substitutions as defined in Tables 12 to 14. In a preferred embodiment, the invention relates to an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to the invention, wherein: (a) the heavy chain variable domain of the antibody comprises 4 heavy chain framework regions, HFR1 to HFR4, and 3 complementarity determining regions HCDR1 to HCDR3 that are operably linked in the order HFR1-HCDR1-HFR2-HCDR2-HFR3-HCDR3-HFR4, wherein each of the heavy chain framework regions has at least 90%, at least

91%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99% or preferably has 100% amino acid identity with the framework amino acid sequence of SEQ ID NO 8, or wherein HFR1 to HFR4 differ from SEQ ID NO 8 in one or more of the amino acid substitutions as defined in Tables 8 to 11; and (b) the light chain variable domain of the antibody comprises 4  
 5 light chain framework regions, LFR1 to LFR4, and 3 complementarity determining regions LCDR1 to LCDR3 that are operably linked in the order LFR1-LCDR1-LFR2-LCDR2-LFR3-LCDR3-LFR4, wherein each of the light chain framework regions has at least 90%, at least 91%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99% or preferably has 100% amino acid identity with the framework amino acid sequence of SEQ ID NO 9, or wherein  
 10 LFR1, LFR2 and/or LFR4 differ from SEQ ID NO 9 in one or more amino acid substitutions as defined in Tables 12 to 14.

Preferably, the amino acid residues that are present at each position (according to Kabat numbering) of the FR1, FR2, FR3 and FR4 of the variable domain of the heavy chain are as indicated in Tables 8 to 11 for FR1, FR2, FR3 and FR4, respectively, or a conservative amino  
 15 acid substitution thereof, and the amino acid residues that are present at each position (according to Kabat numbering) of the FR1, FR2, and FR4 of the variable domain of the light chain preferably are as indicated in Tables 12 to 14 for FR1, FR2, and FR4, respectively or a conservative amino acid substitution thereof. More preferably, however, the framework amino acid residue for any position within the framework region is selected from the amino acid  
 20 residues shown in the corresponding position in Tables 8 to 14.

An anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to the invention, preferably comprises HCDR, LCDR and heavy chain and light chain framework regions selected from the group consisting of: (a) HCDR1 comprises SEQ ID NO:36; HCDR2 comprises SEQ ID NO:44; HCDR3 comprises SEQ ID NO:45; LCDR1 comprises SEQ ID  
 25 NO:39; LCDR2 comprises SEQ ID NO:40; LCDR3 comprises SEQ ID NO:41; HFR1 to HFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NO:8, or wherein HFR1 to HFR4 differ from SEQ ID NO:8 in one  
 30 or more amino acid substitutions as defined in Tables 8 to 11; LFR1 to LFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NO:9, or wherein LFR1, LFR2 and/or LFR4 differ from SEQ ID NO:9 in one or more amino

acid substitutions as defined in Tables 12 to 14; (b) HCDR1 comprises SEQ ID NO:30; HCDR2 comprises SEQ ID NO:31; HCDR3 comprises SEQ ID NO:32; LCDR1 comprises SEQ ID NO:33; LCDR2 comprises SEQ ID NO:34; LCDR3 comprises SEQ ID NO:35; HFR1 to HFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of any one of SEQ ID NOs 1, 3, 4, 5, 14 or 15, or wherein HFR1 to HFR4 differ from any one of SEQ ID NOs 1, 3, 4, 5, 14 or 15 in one or more amino acid substitutions as defined in Tables 8 to 11; LFR1 to LFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NO:2, or wherein LFR1, LFR2 and/or LFR4 differ from SEQ ID NO:2 in one or more amino acid substitutions as defined in Tables 12 to 14; (c) HCDR1 comprises SEQ ID NO:36; HCDR2 comprises SEQ ID NO:37; HCDR3 comprises SEQ ID NO:38; LCDR1 comprises SEQ ID NO:39; LCDR2 comprises SEQ ID NO:40; LCDR3 comprises SEQ ID NO:46; HFR1 to HFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NOs 6 or 13, or wherein HFR1 to HFR4 differ from SEQ ID NOs 6 or 13 in one or more amino acid substitutions as defined in Tables 8 to 11; LFR1 to LFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NO:7, or wherein LFR1, LFR2 and/or LFR4 differ from SEQ ID NO:7 in one or more amino acid substitutions as defined in Tables 12 to 14; (d) HCDR1 comprises SEQ ID NO:36; HCDR2 comprises SEQ ID NO:37; HCDR3 comprises SEQ ID NO:38; LCDR1 comprises SEQ ID NO:39; LCDR2 comprises SEQ ID NO:40; LCDR3 comprises SEQ ID NO:41; HFR1 to HFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NOs: 6, 10 or 11, or wherein HFR1 to HFR4 differ from any one of SEQ ID NOs 6, 10 or 11 in one or more amino acid substitutions as defined in Tables 8 to 11; LFR1 to LFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at

least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NO:9, or wherein LFR1, LFR2 and/or LFR4 differ from SEQ ID NO:9 in one or more amino acid substitutions as defined in Tables 12 to 14; and (e) HCDR1 comprises SEQ ID NO:36; HCDR2 comprises SEQ ID NO:42; HCDR3 comprises SEQ ID NO:43; LCDR1 comprises SEQ ID NO:39; LCDR2 comprises SEQ ID NO:40; LCDR3 comprises SEQ ID NO:41; HFR1 to HFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NO:12, or wherein HFR1 to HFR4 differ from SEQ ID NO:12 in one or more amino acid substitutions as defined in Tables 8 to 11; LFR1 to LFR4 having at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NO:9, or wherein LFR1, LFR2 and/or LFR4 differ from SEQ ID NO:9 in one or more amino acid substitutions as defined in Tables 12 to 14. More preferably, an anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to the invention, comprises: (a) HCDR1 comprising SEQ ID NO:36; HCDR2 comprising SEQ ID NO:44; HCDR3 comprising SEQ ID NO:45; LCDR1 comprising SEQ ID NO:39; LCDR2 comprising SEQ ID NO:40; and LCDR3 comprising SEQ ID NO:41; wherein (i) HFR1 to HFR4 have at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NO:8, or wherein HFR1 to HFR4 differ from SEQ ID NO:8 in one or more amino acid substitutions as defined in Tables 8 to 11; and (ii) LFR1 to LFR4 have at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% or most preferably 100% amino acid identity with the framework amino acid sequences of SEQ ID NO:9, or wherein LFR1, LFR2 and/or LFR4 differ from SEQ ID NO:9 in one or more amino acid substitutions as defined in Tables 12 to 14.

Alternatively or in combination with a previous embodiment, in a preferred embodiment, the framework substitutions are limited to the residues provided in Tables 8 to 14. Thus, it is preferred that no substitutions are made at other locations within the framework regions and it

is also preferred that substitutions are limited to the residues shown in Tables 8 to 14 or a conservative amino acid substitution thereof.

Alternatively or in combination with a previous embodiment, in a preferred embodiment an anti-SIRP $\alpha$  antibody according to the present invention or an antigen-binding fragment thereof comprises a HCVR and a LCVR selected from the group consisting of: (a) HCVR amino acid sequence of SEQ ID NO:8; LCVR amino acid sequence of SEQ ID NO:9; (b) HCVR amino acid sequence of SEQ ID NO:1; LCVR amino acid sequence of SEQ ID NO:2; (c) HCVR amino acid sequence of SEQ ID NO:3; LCVR amino acid sequence of SEQ ID NO:2; (d) HCVR amino acid sequence of SEQ ID NO:4; LCVR amino acid sequence of SEQ ID NO:2; (e) HCVR amino acid sequence of SEQ ID NO:5; LCVR amino acid sequence of SEQ ID NO:2; (f) HCVR amino acid sequence of SEQ ID NO:6; LCVR amino acid sequence of SEQ ID NO:7; (g) HCVR amino acid sequence of SEQ ID NO:10; LCVR amino acid sequence of SEQ ID NO:9; (h) HCVR amino acid sequence of SEQ ID NO:6; LCVR amino acid sequence of SEQ ID NO:9; (i) HCVR amino acid sequence of SEQ ID NO:11; LCVR amino acid sequence of SEQ ID NO:9; (j) HCVR amino acid sequence of SEQ ID NO:12; LCVR amino acid sequence of SEQ ID NO:9; (k) HCVR amino acid sequence of SEQ ID NO:13; LCVR amino acid sequence of SEQ ID NO:7; (l) HCVR amino acid sequence of SEQ ID NO:14; LCVR amino acid sequence of SEQ ID NO:2; and (m) HCVR amino acid sequence of SEQ ID NO:15; LCVR amino acid sequence of SEQ ID NO:2. More preferably, an anti-SIRP $\alpha$  antibody according to the present invention or an antigen-binding fragment thereof comprises a HCVR and a LCVR selected from the group consisting of: (a) HCVR amino acid sequence of SEQ ID NO:8 and LCVR amino acid sequence of SEQ ID NO:9; (b) HCVR amino acid sequence of SEQ ID NO:1 and LCVR amino acid sequence of SEQ ID NO:2; (c) HCVR amino acid sequence of SEQ ID NO:3 and LCVR amino acid sequence of SEQ ID NO:2; (d) HCVR amino acid sequence of SEQ ID NO:4 and LCVR amino acid sequence of SEQ ID NO:2; (e) HCVR amino acid sequence of SEQ ID NO:5 and LCVR amino acid sequence of SEQ ID NO:2; (f) HCVR amino acid sequence of SEQ ID NO:14 and LCVR amino acid sequence of SEQ ID NO:2; and (g) HCVR amino acid sequence of SEQ ID NO:15 and LCVR amino acid sequence of SEQ ID NO:2. Most preferably, an anti-SIRP $\alpha$  antibody according to the present invention or an antigen-binding fragment thereof comprises a HCVR amino acid sequence of SEQ ID NO:8 and a LCVR amino acid sequence of SEQ ID NO:9.

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

relevant animal model. Affinity of the antibodies according to the invention for SIRP $\alpha$  from other species is not excluded.

Without wishing to be bound by any theory, it is believed that an antibody or antigen-binding fragment thereof according to the invention may act by one of the following. An antibody of the invention may bind to the same site where CD47 binds, preventing binding of SIRP $\alpha$  by CD47 and consequently inhibiting the signalling that negatively regulates the Fc-receptor-dependent action of immune effector cells.

The anti-SIRP $\alpha$  antibodies or antigen-binding fragments thereof according to the invention preferably are more specific than known anti-SIRP $\alpha$  antibodies, and show excellent affinity for both human SIRP $\alpha_{\text{BII}}$  and human SIRP $\alpha_{\text{I}}$ , while they have reduced, more preferably low affinity, even more preferably no detectable affinity for human SIRP $\gamma$ , preferably as measured using human CD3<sup>+</sup> T-cell staining according to the Examples.

In some embodiments, an anti-SIRP $\alpha$  antibody of the invention comprises a light chain and/or a heavy chain antibody constant region. Any antibody constant regions known in the art can be used. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, e.g., a human kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region, e.g., a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. In one embodiment, the light or heavy chain constant region is a fragment, derivative, variant, or mutein of a naturally occurring constant region.

In one particular embodiment, an anti-SIRP $\alpha$  antibody according to the invention comprises an Fc region that binds to activating Fc receptors present on human immune effector cells. Such an anti-SIRP $\alpha$  antibody may be suitable for monotherapy of SIRP $\alpha$ -positive tumours, preferably renal cell carcinoma or melanoma, as it can induce ADCC and/or ADCP (Yanagita *et al.* *JCI Insight* 2017, 2(1), e89140). Without wishing to be bound by any theory, it is believed that destruction of cancer cells by such an antibody occurs, at least in part, through ADCC. Human immune effector cells possess a variety of activating Fc receptors, which upon ligation trigger phagocytosis, trogoptosis, perforin and granzyme release, cytokine release, ADCC, ADCP, ADAP and/or other mechanisms. Examples of these receptors are Fc $\gamma$  receptors, e.g. Fc $\gamma$ RI (CD64), Fc $\gamma$ RIIA (CD32a), Fc $\gamma$ RIIIA (CD16a), Fc $\gamma$ RIIIB (CD16b), Fc $\gamma$ RIIC (CD32c) and the Fc $\alpha$  receptor Fc $\alpha$ RI (CD89). The various natural antibody isotypes bind to a variety of these receptors. E.g. IgG<sub>1</sub> binds to Fc $\gamma$ RI, Fc $\gamma$ RIIA, Fc $\gamma$ RIIC, Fc $\gamma$ RIIIA, Fc $\gamma$ RIIIB; 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 $\gamma$ RIIIB; IgG<sub>4</sub> binds to Fc $\gamma$ RI, Fc $\gamma$ RIIA, Fc $\gamma$ RIIC, Fc $\gamma$ RIIIA; and IgA binds to Fc $\alpha$ RI.

In a preferred embodiment, an 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  
5 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, preferably as shown in SEQ ID NO:24.

Although the anti-SIRP $\alpha$  antibodies comprising an Fc region that binds to activating Fc receptors present on human immune effector cells are suitable to treat cancers expressing CD47  
10 in a combination therapy with a therapeutic antibody, *in vitro* ADCC experiments of a chimeric anti-SIRP $\alpha$  IgG<sub>1</sub> antibody in combination with a therapeutic antibody (trastuzumab) comprising a human Fc region that binds to activating Fc receptors present on human immune effector cells (i.e. antibodies that are able to induce ADCC and/or ADAP) did not show the increase in ADCC as had been expected on the basis of earlier results using murine antibodies (Figure 2). Without  
15 wishing to be bound by any theory, it is believed that the Kurlander effect may occur (Kurlander *J Immunol* 1983, 131(1), 140-147), for the anti-SIRP $\alpha$  antibody, which may compete with the therapeutic antibody (in this case trastuzumab) for binding to the activating Fc receptor on the immune effector cell (in this case the neutrophilic granulocyte). Thus, Fc tail of the anti-SIRP $\alpha$  may bind in *cis* to the Fc receptor on the relevant phagocytic immune effector cell e.g.  
20 granulocyte, macrophage or dendritic cell thereby reducing or preventing ADCC and/or ADAP. Consequently, reduction of binding of the Fc region of the anti-SIRP $\alpha$  antibody to the activating Fc receptors present on human immune effector cells, for instance by modifying the Fc tail, was shown to improve ADCC by a therapeutic antibody (trastuzumab; Figure 2). Therefore, in a preferred embodiment, the present invention relates to an anti-SIRP $\alpha$   
25 antibody that exhibits reduced binding to and/or low affinity for activating Fc receptors present on human immune effector cells and that can be advantageously used in a combination therapy with a therapeutic antibody as is further explained in section "Use of the antibodies of the invention or fragments thereof". Such an anti-SIRP $\alpha$  antibody may comprise a native Fc region that has a low affinity for the Fc receptor, such as for example IgG<sub>4</sub> or IgG<sub>2</sub> Fc, or such an anti-  
30 SIRP $\alpha$  antibody may 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. Alternatively, enzymatic deglycosylation reduces binding of the Fc to Fc gamma receptors, without requiring amino acid mutations. Reduced binding in this context means that the affinity of the anti-SIRP $\alpha$  antibody comprising a modified Fc region for the activating Fc receptors is

less than the affinity of an anti-SIRP $\alpha$  antibody with the same variable regions comprising a similar unmodified Fc region. Preferably, the affinity decreases at least 2-fold, preferably at least 3-fold, preferably at least 4-fold, preferably at least 5-fold, preferably at least 10-fold, preferably at least 50-fold, preferably at least 100-fold, preferably at least 1000-fold. The binding affinity of antibodies for activating Fc receptors is typically measured using 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. Anti-SIRP $\alpha$  antibodies exhibiting reduced binding to or low affinity for the human Fc $\alpha$  or Fc $\gamma$  receptor in combination with a therapeutic antibody are especially effective in cellular destruction of cancer cells by increasing ADCC and/or ADCP and/or ADAP of immune effector cells. Typically, the Fc region of an anti-SIRP $\alpha$  antibody according to the invention is modified to reduce binding to activating Fc receptors present on human immune effector cells.

In a preferred embodiment, an anti-SIRP $\alpha$  antibody according to the invention comprises a modified Fc region that exhibits reduced binding to or low affinity for, preferably no binding to, a human Fc $\alpha$  or Fc $\gamma$  receptor. For instance, 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); 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/or P331S; or H268Q, V309L, A330S, and/or P331S (numbering analogue to IgG<sub>1</sub> Eu numbering) (Vafa *et al. Methods* 2014, 65, 114-126); IgG<sub>3</sub> binding can be reduced by introducing e.g. amino acid substitutions L234A and L235A, or amino acid substitutions L234A, L235A and P331S (Leoh *et al. Mol. Immunol.* 2015, 67, 407-415); and IgG<sub>4</sub> binding can be reduced by introducing e.g. amino acid substitutions S228P, F234A and/or 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/or P440R (sequential numbering, Pleass *et al. J. Biol. Chem.* 1999, 271(33), 23508-23514).

Preferably, an anti-SIRP $\alpha$  antibody according to the invention comprises a modified Fc region that exhibits reduced binding to or low affinity for, preferably no binding to, a human Fc $\alpha$  or Fc $\gamma$  receptor compared to the same anti-SIRP $\alpha$  antibody comprising a wild-type Fc region. 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, an 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).

In a preferred embodiment, an anti-SIRP $\alpha$  antibody of the invention comprises a modified human IgG<sub>1</sub> Fc region comprising 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. More 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. Alternatively preferred, the one or more amino acid substitutions are selected from the group consisting of L234A, L234E, L235A, G237A, D265A, D265E, D265N, D270A, D270E, D270N, A327Q, P328A, P329A and P329G. Even more preferably, an anti-SIRP $\alpha$  antibody of the invention comprises a modified human IgG<sub>1</sub> Fc region comprising one or more amino acid substitutions selected from the group consisting of L234A, L234E, L235A, G237A, D265A, D265E, D265N, P328A, P329A and P329G. In a preferred embodiment, the modified human IgG<sub>1</sub> Fc region comprises amino acid substitutions: (i) L234A and L235A; (ii) L234E and L235A; (iii) L234A, L235A and P329A; or (iv) L234A, L235A and P329G. More preferably, the modified human IgG<sub>1</sub> Fc region comprises amino acid substitutions: (i) L234A and L235A; or (ii) L234E and L235A. Most preferably, the modified human IgG<sub>1</sub> Fc region comprises amino acid substitutions L234A and L235A. In combination with any one of the previous embodiments, in a preferred embodiment 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. Thus, it is preferred that the amino acid residue at position 297 according to Eu numbering is asparagine. An anti-SIRP $\alpha$  antibody according to this embodiment, preferably comprises a modified Fc region of the IgG<sub>1</sub> genotype as shown in SEQ ID NO:25 (with L234A and L235A mutations shown at positions 117 and 118).

#### Production and purification of the antibodies of the invention or fragments thereof

Anti-SIRP $\alpha$  antibodies of the invention or antigen-binding fragments thereof can be prepared by any of a number of conventional techniques. They will usually be produced in recombinant expression systems, using any technique known in the art. See e.g. Shukla and Thömmes (*Trends in Biotechnol.* 2010, 28(5), 253-261), Harlow and Lane. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988, and Sambrook and Russell. *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, NY, 2001. Any expression system

known in the art can be used to make the recombinant polypeptides of the invention. In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a desired polypeptide.

In one aspect, the invention therefore relates to a nucleic acid molecule comprising a nucleotide sequence encoding an anti-SIRP $\alpha$  antibody of the invention or an antigen-binding fragment thereof. One nucleotide sequence encodes a polypeptide comprising at least the variable domain of the light chain of an anti-SIRP $\alpha$  antibody of the invention; another nucleotide sequence encodes a polypeptide comprising at least the variable domain of the heavy chain of an anti-SIRP $\alpha$  antibody of the invention. Thus, in a preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding at least one of the HCVR and the LCVR of the antibody. A preferred nucleic acid molecule is an expression vector wherein the nucleotide sequences encoding the antibody polypeptides of the invention are operably linked to expression regulatory sequences, such as e.g. a promoter and a leader sequence (also referred to as a signal peptide, signal sequence, targeting signal, localization signal, localization sequence, transit peptide or leader peptide), for expression of the coding nucleotide sequence in a host cell. A preferred leader sequence for the heavy chain is shown in SEQ ID NO:28. A preferred leader sequence for the light chain is shown in SEQ ID NO:29. Another suitable leader sequence for use in the present invention is shown in SEQ ID NO:27.

In another aspect, the invention pertains to a host cell comprising a nucleic acid molecule as defined above in this section. The cell preferably is an isolated cell or a cultured cell. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram positive organisms, for example *Escherichia coli* or bacilli. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include Chinese Hamster Ovary (CHO) cells, COS-7 line of monkey kidney cells (Gluzman *et al. Cell* 1981, 23, 175), human embryonic kidney (HEK) 293 cells, L cells, C127 cells, 3T3 cells, HeLa cells, baby hamster kidney (BHK) cell lines, and the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 as described by McMahan *et al. EMBO J.* 1991, 10, 2821. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are for example described by Pouwels *et al* (Cloning Vectors: A Laboratory Manual, Elsevier, New York, 1985).

The transformed cells can be cultured under conditions that promote expression of the polypeptide. Thus in one aspect, the invention relates to a method for producing an anti-SIRP $\alpha$  antibody of the invention or antigen-binding fragment thereof, the method comprising the step

of cultivating a cell comprising at least one expression vector as defined herein, under conditions conducive to expression of the polypeptide and, optionally, recovering the polypeptide.

5 An anti-SIRP $\alpha$  antibody according to the invention or antigen-binding fragment thereof can be recovered by conventional protein purification procedures, including e.g. hydroxyapatite chromatography, gel electrophoresis, dialysis, affinity chromatography (such as for example protein A-Sepharose, protein G-Sepharose), ion exchange chromatography (such as for example anion-exchange chromatography, cation-exchange chromatography, mixed mode), or hydrophobic interaction chromatography (see e.g. Low *et al. J. Chromatography B* 2007, 848, 10 48-63; Shukla *et al. J. Chromatography B* 2007, 848, 28-39). Affinity chromatography encompasses affinity chromatography using CaptureSelect™ ligands, which offer a unique affinity purification solution based on Camelid-derived single domain (VHH) antibody fragments (see e.g. Eifler *et al. Biotechnology Progress* 2014, 30(6), 1311-1318). Polypeptides contemplated for use herein include substantially homogeneous recombinant anti-SIRP $\alpha$  15 antibody polypeptides substantially free of contaminating endogenous materials.

Amino acid sequence modification(s) of the anti-SIRP $\alpha$  antibody of the invention or antigen-binding fragment thereof are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody are prepared by introducing appropriate nucleotide changes 20 into the antibody encoding nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody or antigen-binding fragment thereof. Any combination of deletion, insertion, and substitution is made to achieve the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes may also alter 25 post-translational processes of the antibody or antigen-binding fragment thereof, such as changing the number or position of glycosylation sites.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal 30 insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody or the antigen-binding fragment thereof include the fusion with an enzyme or a polypeptide which increases the serum half-life of the antibody or antigen-binding fragment.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody or the antigen-binding fragment thereof replaced by a different residue. Such substitutional mutagenesis of antibodies or antigen-binding fragments thereof include FR alterations as indicated above as well as alterations to reduce binding of the Fc region to an Fc receptor to prevent receptor activation as indicated above.

Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody or the antigen-binding fragment thereof. By altering is meant deleting one or more carbohydrate moieties found in the antibody or the antigen-binding fragment thereof, and/or adding one or more glycosylation sites that are not present in the antibody or the antigen-binding fragment thereof. Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of any of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the monosaccharides or monosaccharide derivatives N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites). Nucleic acid molecules encoding amino acid sequence variants of the antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody or the antigen-binding fragment thereof.

### Compositions comprising the antibodies of the invention or fragments thereof

In another aspect, the invention relates to a pharmaceutical composition comprising an anti-SIRP $\alpha$  antibody of the invention or an antigen-binding fragment thereof, or a pharmaceutical derivative or prodrug thereof, together with one or more pharmaceutically acceptable excipients, such as for example a pharmaceutically acceptable carrier, an adjuvant,

or a vehicle. Preferably, the invention relates to a pharmaceutical composition comprising an anti-SIRP $\alpha$  antibody of the invention, or an antigen-binding fragment thereof, and a pharmaceutically acceptable excipient.

Such a pharmaceutical composition is for administration to a subject. A pharmaceutical  
5 composition according to the invention can be used in the methods of treatment described hereinbelow by administration of an effective amount of the composition to a subject in need thereof. The term “subject” as used herein refers to all animals classified as mammals and includes, but is not restricted to, primates and humans. The subject is preferably a human.

The term “pharmaceutically acceptable excipient” as used herein, is intended to include  
10 any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and adsorption delaying agents, and the like, compatible with pharmaceutical administration (see e.g. Handbook of Pharmaceutical Excipients, Rowe *et al.* Eds. 7th edition, 2012, www.pharmpress.com). The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is  
15 incompatible with the active compound, use thereof in the composition is contemplated. Acceptable excipients, including carriers or stabilizers, are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, acetate, histidine, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride;  
20 benzalkonium chloride, benzethonium chloride; phenol, butyl, or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine,  
25 or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polysorbate (e.g. TWEEN<sup>TM</sup>), poloxamer (e.g. PLURONICS<sup>TM</sup>), hydroxypropyl- $\beta$ -cyclodextrin or polyethylene glycol  
30 (PEG).

Supplementary active compounds can also be incorporated into the pharmaceutical composition of the invention. Thus, in a particular embodiment, the pharmaceutical composition of the invention may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do

not adversely affect each other. The effective amount of such other active agent depends, among other things, on the amount of anti-SIRP $\alpha$  antibody of the invention or antigen-binding fragment thereof present in the pharmaceutical composition, the type of disease or disorder or treatment, etc.

5        In an embodiment, the anti-SIRP $\alpha$  antibody of the invention or the antigen-binding fragment thereof is prepared with carriers that will protect said compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems, e.g. liposomes. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen,  
10 polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions, including targeted liposomes can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in US Patent No. 4,522,811 or WO2010/095940.

15        The administration route of the anti-SIRP $\alpha$  antibody of the invention, or antigen-binding fragment thereof, can be oral, parenteral, by inhalation or topical. The term “parenteral” as used herein includes intravenous, intra-arterial, intralymphatic, intraperitoneal, intramuscular, subcutaneous, rectal or vaginal administration. The intravenous forms of parenteral administration are preferred. By “systemic administration” is meant oral, intravenous,  
20 intraperitoneal and intramuscular administration. The amount of an antibody required for therapeutic or prophylactic effect will, of course, vary with the antibody chosen, the nature and severity of the condition being treated and the subject. In addition, the antibody may suitably be administered by pulse infusion, e.g., with declining doses of the antibody. Preferably, the dosing is given by injections, most preferably intravenous or subcutaneous injections,  
25 depending in part on whether the administration is brief or chronic.

Thus, in a particular embodiment, the pharmaceutical composition of the invention may be in a form suitable for parenteral administration, such as sterile solutions, suspensions or lyophilized products in the appropriate unit dosage form. Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and  
30 sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, CremophorEM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against

the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid polyethylene glycol, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating  
5 such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, or sodium chloride in the  
10 composition.

Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminium monostearate or gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a  
15 polypeptide or antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable  
20 solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

In a particular embodiment, said pharmaceutical composition is administered via intravenous (IV) or subcutaneous (SC) route. Adequate excipients can be used, such as bulking  
25 agents, buffering agents or surfactants. The mentioned formulations will be prepared using standard methods for preparing parenterally administrable compositions as are well known in the art and described in more detail in various sources, including, for example, Remington: The Science and Practice of Pharmacy. Allen Ed. 22nd edition, 2012, [www.pharmpress.com](http://www.pharmpress.com)).

It is especially advantageous to formulate the pharmaceutical compositions, namely  
30 parenteral compositions, in dosage unit form to ease administration and for uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound (anti-SIRP $\alpha$  antibody of the invention or antigen-binding fragment thereof) calculated to produce the desired therapeutic effect in association with the required

pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

5           Generally, an effective administered amount of an anti-SIRP $\alpha$  antibody of the invention will depend on the relative efficacy of the compound chosen, the severity of the disorder being treated and the weight of the sufferer. However, active compounds will typically be administered once or more times a day for example 1, 2, 3 or 4 times daily, with typical total daily doses in the range of from 0.001 to 1,000 mg/kg bodyweight/day, preferably about 0.01  
10 to about 100 mg/kg bodyweight/day, most preferably from about 0.05 to 10 mg/kg bodyweight/day. Guidance in selecting appropriate doses of antibodies is available (see, e.g., Wawrzynczak (1996) *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.) (1991) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, N.Y.; Bach (ed.) (1993) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*,  
15 Marcel Dekker, New York, N.Y.; Baert *et al.* (2003) *New Engl. J. Med.* 1999, 348, 601-608; Milgrom *et al.* *New Engl. J. Med.* 1999, 341, 1966-1973; Slamon *et al.* *New Engl. J. Med.* 2001, 344, 783-792; Beniaminovitz *et al.* *New Engl. J. Med.* 2000, 342, 613-619; Ghosh *et al.* *New Engl. J. Med.* 2003, 348, 24-32; Lipsky *et al.* *New Engl. J. Med.* 2000, 343, 1594-1602).

Determination of the appropriate dose is made by the clinician, e.g., using parameters or  
20 factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, e.g., the inflammation or level of inflammatory cytokines produced. Antibodies or antibody  
25 fragments can be administered by continuous infusion, or by doses at intervals of, e.g., one day, one week, or 1-7 times per week. Alternatively, antibodies or antibody fragments can be administered once daily, every other day, 2-3 times weekly, once every 2 weeks, once every 3 weeks, once every 6 weeks. A preferred dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects. A total or mean weekly dose is  
30 generally at least 0.05  $\mu$ g/kg body weight, more generally at least 0.2  $\mu$ g/kg, most generally at least 0.5  $\mu$ g/kg, typically at least 1  $\mu$ g/kg, more typically at least 10  $\mu$ g/kg, most typically at least 100  $\mu$ g/kg, preferably at least 0.2 mg/kg, more preferably at least 1.0 mg/kg, most preferably at least 2.0 mg/kg, optimally at least 10 mg/kg, more optimally at least 25 mg/kg, and most optimally at least 50 mg/kg (see, e.g., Yang *et al.* *New Engl. J. Med.* 2003 349, 427-

434; Herold *et al.* *New Engl. J. Med.* 2002, 346, 1692-1698; Liu *et al.* *J. Neurol. Neurosurg. Psych.* 1999, 67, 451-456; Portielje *et al.* *Cancer Immunol. Immunother.* 2003, 52, 133-144). Preferably, the total or mean weekly dose is in the range of from 0.001 to 100 mg/kg bodyweight, preferably about 0.01 to about 50 mg/kg bodyweight, preferably about 0.05 to about 30 mg/kg bodyweight, most preferably from about 0.1 to 10 mg/kg bodyweight.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

The anti-SIRP $\alpha$  antibodies of the present invention or antigen-binding fragments thereof are preferably used in combination with a therapeutic antibody as will be detailed more in the following section and possibly in combination with other drugs. The therapeutic antibody may form part of the same composition, or be provided as a separate composition for administration at the same time or at a different time. Alternatively or in combination with any of the previous embodiments, any other drugs may form part of the same composition or be provided as a separate composition for administration at the same time or at a different time.

Preferably, a pharmaceutical composition of the invention is in the form of a lyophilized cake (lyophilized powders), which requires (aqueous) dissolution (i.e. reconstitution) before intravenous administration, or of a frozen (aqueous) solution, which requires thawing before administration. Most preferably, the pharmaceutical composition is in the form of a lyophilized cake. Suitable pharmaceutically acceptable excipients for inclusion into the pharmaceutical composition (before freeze-drying) in accordance with the present invention include buffer solutions (e.g. citrate, acetate, histidine or succinate containing salts in water), lyoprotectants (e.g. sucrose, trehalose), tonicity modifiers (e.g. sodium chloride), surfactants (e.g. polysorbate or hydroxypropyl- $\beta$ -cyclodextrin), and bulking agents (e.g. mannitol, glycine). Excipients used for freeze-dried protein formulations are selected for their ability to prevent protein denaturation during the freeze-drying process as well as during storage.

#### Use of the antibodies of the invention or fragments thereof

The anti-SIRP $\alpha$  antibodies, antigen-binding fragments thereof and pharmaceutical compositions of the invention will be useful in the treatment of diseases, conditions and indications where SIRP $\alpha$  is expressed or where SIRP $\alpha$  and CD47 are expressed, preferably overexpressed, in particular for the treatment of CD47-expressing cancer.

Thus, in a further aspect, the present invention relates to an anti-SIRP $\alpha$  antibody of the invention or an antigen-binding fragment thereof, or a pharmaceutical composition of the invention, for use as a medicament.

In another aspect, the present invention relates to an anti-SIRP $\alpha$  antibody of the invention or an antigen-binding fragment thereof, or a pharmaceutical composition of the invention for use in the treatment of a cancer, preferably in the treatment of CD47-expressing cancer.

CD47 has been found to be expressed on several human tumour types, including acute  
5 myeloid leukaemia (AML), breast cancer, chronic myeloid leukaemia (CML), chronic  
lymphatic leukaemia (CLL), acute lymphoblastic leukaemia (ALL), non-Hodgkin's lymphoma  
(NHL), including follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL),  
hepatocellular carcinoma, multiple myeloma (MM), bladder cancer, colon cancer, gastric  
cancer, ovarian cancer, head and neck cancer, neuroblastoma, melanoma, osteosarcoma,  
10 pancreatic cancer, renal carcinoma, prostate cancer, hepatocellular carcinoma, lung cancer and  
other solid tumours (Chao *et al. Curr Opin Immunol.* 2012, 24(2), 225-232; Chao *et al. Cell*  
2010, 142(5), 699-713; Rösner *et al. Mol Cancer Ther.* 2018). Increased expression has been  
observed for several of these tumours as compared to their normal cell counterparts (Russ *et al.*  
*Blood Rev.* 2018, doi: 10.1016/j.blre.2018.04.005). It has been hypothesized that – along with  
15 other hypothesized CD47-mechanisms – CD47 upregulation in tumour cells enables the  
tumours to escape innate immune system surveillance through evasion of phagocytosis (Chao  
*et al. Curr Opin Immunol.* 2012, 24(2), 225-232).

Interestingly, Yanagita *et al.* reported that human renal cell carcinoma and melanoma  
highly express SIRP $\alpha$  (Yanagita *et al. JCI Insight* 2017, 2(1), e89140). In addition, SIRP $\alpha$   
20 expression has been found on some neuroblastoma cells and in acute myeloid leukaemia  
(AML). Sosale *et al.* reported SIRP $\alpha$  expression in lung carcinoma and glioblastoma (Sosale *et al.*  
*Mol. Ther. Methods Clin. Dev.* 2016, 3, 16080). Chen *et al.* reported SIRP $\alpha$  expression on  
astrocytoma and glioblastoma (Chen *et al. Cancer Res* 2004, 64(1), 117-127). Mesothelioma  
and B-cell lymphoma can also be SIRP $\alpha$ -positive. Thus, in another aspect, the present invention  
25 relates to an anti-SIRP $\alpha$  antibody of the invention or an antigen-binding fragment thereof or a  
pharmaceutical composition of the invention for use in the treatment of a disease, condition or  
indication where SIRP $\alpha$  is expressed, in particular in the treatment of SIRP $\alpha$ -expressing cancer,  
such as for example human renal cell carcinoma and melanoma, but also in autoimmune disease  
such as for example in rheumatoid arthritis, multiple sclerosis and perhaps granulomatosis with  
30 polyangiitis (GPA), microscopic polyangiitis (MPA) and pemphigus vulgaris (PV). Anti-SIRP $\alpha$   
antibodies could also increase efficacy of another antibody in diseases whether this latter  
antibody is used to deplete pathogenic or infected cells. For a SIRP $\alpha$ -expressing tumour, an  
anti-SIRP $\alpha$  antibody of the invention comprising wild-type human Fc may be suitable as  
monotherapy. In one 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 in the treatment of SIRP $\alpha$ -positive human solid tumours and haematological malignancies, preferably renal cell carcinoma or malignant melanoma. Preferably, the Fc region that binds to activating Fc receptors present on human immune effector cells is of the IgA or IgG isotype.

5 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.

As indicated above, anti-SIRP $\alpha$  antibodies of the invention or antigen-binding fragments thereof wherein preferably the Fc effector function is partly or completely disrupted, can be used to improve the effector functions – such as for example increase ADCC – of a therapeutic antibody. Preferably, a cancer to be treated with such an antibody of the invention or antigen-binding fragment thereof and a therapeutic antibody does not express SIRP $\alpha$ . Such a method of treatment is preferably combined with one or more further anti-cancer therapies. An anti-SIRP $\alpha$  antibody 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 was found to enhance the *in vitro* ADCC of a therapeutic antibody using neutrophils as effector cells. The antibodies 1 – 13 provided in the Examples show a dose-dependent increase in the *in vitro* ADCC using neutrophils of heterozygous SIRP $\alpha_1$ /SIRP $\alpha_{BIT}$  donors. Preferred antibodies are the ones that increase *in vitro* ADCC using neutrophils to the best extent while preferably not showing signs of immunogenicity *in vitro* using T-cell proliferation assay and/or IL-2 ELISpot. Most preferred are antibodies 1 – 6, 12 and 13, preferably antibody 6.

Preferably, the therapeutic antibody is an antibody approved by a medicines regulatory authority, such as the European Medicines Agency (EMA) or Food and Drug Administration (FDA). Online databases of most Regulatory Authorities can be consulted to find whether an antibody is approved.

Typically, the therapeutic antibody for use in combination with an anti-SIRP $\alpha$  antibody according to the invention (preferably with reduced binding of its Fc region) or an antigen-binding fragment thereof, is a monospecific or bispecific antibody or antibody fragment comprising at least one of an HCVR and a LCVR binding to a target selected from the group consisting of annexin A1, AMHR2, AXL, BCMA, B7H3, B7H4, CA6, CA9, CA15-3, CA19-9, CA27-29, CA125, CA242, CCR2, CCR4, CCR5, CD2, CD4, CD16, CD19, CD20, CD22, CD27, CD30, CD33, CD37, CD38, CD40, CD44, CD47, CD52, CD56, CD70, CD74, CD79, CD98, CD115, CD123, CD138, CD203c, CD303, CD333, CEA, CEACAM, CLCA-1, CLL-1,

c-MET, Cripto, CTLA-4, DLL3, EGFL, EGFR, EPCAM, Eph (e.g. EphA2 or EphB3), endothelin B receptor (ETBR), FAP, FcRL5 (CD307), FGF, FGFR (e.g. FGFR3), FOLR1, fucosyl-GM1, GCC, GD2, GPNMB, gp100, HER2, HER3, HMW-MAA, integrin  $\alpha$  (e.g.  $\alpha\text{v}\beta 3$  and  $\alpha\text{v}\beta 5$ ), IGF1R, IL1RAP, kappa myeloma antigen, TM4SF1 (or L6 antigen), Lewis A like  
 5 carbohydrate, Lewis X, Lewis Y, LIV1, mesothelin, MUC1, MUC16, NaPi2b, Nectin-4, PD-1, PD-L1, prolactin receptor, PSMA, PTK7, SLC44A4, STEAP-1, 5T4 antigen (or TPBG, trophoblast glycoprotein), TF (tissue factor), Thomsen-Friedenreich antigen (TF-Ag), Tag72, TNF, TNFR, TROP2, VEGF, VEGFR, and VLA.

Non-limiting examples of cancers that express such a target are: (HER2-positive) breast  
 10 cancer, (EGFR-positive) colon carcinoma, (GD2-positive) neuroblastoma, melanoma, osteosarcoma, (CD20-positive) B-cell lymphomas, (CD38-positive) multiple myeloma (CD52-positive) lymphoma, and (CD33-positive) acute myeloid leukaemia (AML).

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

15 In a preferred embodiment, a therapeutic antibody against a membrane-bound target on the surface of tumour cells 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.  
 20 Therapeutic antibodies of the human IgG, IgE, or IgA isotype comprise a human Fc region that binds to activating Fc receptors present on human immune effector cells.

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 antibody  
 25 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.

Suitable therapeutic antibodies for use in combination with an anti-SIRP $\alpha$  antibody according to the invention or an antigen-binding fragment thereof include alemtuzumab (for example in the treatment of multiple sclerosis), , obinutuzumab (for example in treatment of CLL, FL), ofatumumab (for example in treatment of MM), daratumumab (for example in  
 30 treatment of MM), trastuzumab (for example in treatment of HER-2 overexpressing breast cancer, gastric cancer, gastroesophageal junction adenocarcinoma), dinutuximab (for example in treatment of neuroblastoma, paediatric patients), panitumumab, cetuximab (for example in treatment of head&neck cancer, colorectal cancer), rituximab, ofatumumab (for example in treatment of NHL, CLL, FL, DLBCL), ublituximab, margetuximab, pertuzumab, veltuzumab,

brentuximab, elotuzumab, ibritumomab, ifabotuzumab, farletuzumab, otiertuzumab, carotuximab, epratuzumab, inebilizumab, lumretuzumab, mogamulizumab, leukotuximab, isatuximab, oportuzumab, ensituximab, cemiplimab, nivolumab, pembrolizumab, durvalumab, avelumab, atezolizumab, spartalizumab, tislelizumab, camrelizumab, sintilimab, cemiplimab.

5 Such therapeutic antibodies can also be provided as part of an antibody-drug conjugate (ADC). Suitable ADCs for use in combination with an anti-SIRP $\alpha$  antibody according to the invention or an antigen-binding fragment thereof include, but are not limited to, trastuzumab duocarmazine, trastuzumab deruxtecan, trastuzumab emtansine, gemtuzumab ozogamicin, inotuzumab ozogamicin, polatuzumab vedotin, naratuximab emtansine, ibritumomab tiuxetan

10 and brentuximab vedotin.

The antibodies of the invention or antigen-binding fragments thereof and the therapeutic antibody may be in the same formulation or may be administered in different formulations. Administration can be concurrent or sequential, and may be effective in either order.

Thus, in a preferred embodiment, the present invention relates to an anti-SIRP $\alpha$  antibody

15 of the invention or an antigen-binding fragment thereof for use in the treatment of human solid tumours and haematological malignancies in combination with the use of a therapeutic antibody against a membrane-bound target on the surface of tumour cells, which comprises a human Fc region that binds to activating Fc receptors present on human immune effector cells, wherein the anti-SIRP $\alpha$  antibody comprises a modified Fc region that exhibits reduced binding to a

20 human Fc $\alpha$  or Fc $\gamma$  receptor, when compared to the same anti-SIRP $\alpha$  antibody comprising a wild-type Fc region, preferably a modified human IgG<sub>1</sub> Fc region comprising one or more amino acid substitutions at positions selected from the group consisting of: L234, L235, G237, D265, D270, N297, A327, P328, and P329 (Eu numbering).

Alternatively or in combination with any of the other embodiments, in an embodiment,

25 the present invention relates to a use of an anti-SIRP $\alpha$  antibody of the invention or an antigen-binding fragment thereof or a pharmaceutical composition of the invention for the manufacture of a medicament for the treatment of a disease, condition or indication where CD47 is expressed, preferably overexpressed, in particular in the treatment of cancer. For illustrative, non-limitative, cancers to be treated according to the invention: see hereinabove. In a preferred

30 embodiment, the anti-SIRP $\alpha$  antibody of the invention or the antigen-binding fragment thereof or the pharmaceutical composition of the invention are for concurrent or sequential administration with a therapeutic antibody as described above.

Alternatively or in combination with any of the other embodiments, in an embodiment, the present invention relates to a use of an anti-SIRP $\alpha$  antibody of the invention or an antigen-

binding fragment thereof or a pharmaceutical composition of the invention for the manufacture of a medicament for the treatment of a SIRP $\alpha$ -expressing cancer, such as human renal cell carcinoma or melanoma. In a preferred embodiment, the anti-SIRP $\alpha$  antibody of the invention or the antigen-binding fragment thereof or the pharmaceutical composition of the invention are  
5 for concurrent or sequential administration with a therapeutic antibody as described above.

Alternatively or in combination with any of the other embodiments, in an embodiment, the present invention relates to a method for treating cancer, specifically a cancer of which the tumour cells express CD47 or SIRP $\alpha$ , which method comprises administering to a subject in need of said treatment a therapeutically effective amount of an anti-SIRP $\alpha$  antibody of the  
10 invention or an antigen-binding fragment thereof, or a pharmaceutical composition according to the invention. In a particular embodiment, said cancer is a cancer characterized by tumour cells expressing CD47, potentially overexpressing CD47. For illustrative, non-limitative, CD47-expressing cancers to be treated according to the invention: see hereinabove. In an alternative embodiment, the SIRP $\alpha$  expressing cancer is a human renal cell carcinoma or  
15 melanoma or neuroblastoma or acute myeloid leukaemia (AML).

It is preferred that an anti-SIRP $\alpha$  antibody of the invention or an antigen-binding fragment thereof or a composition of the invention inhibits the growth of tumour cells expressing CD47 when used in combination with a therapeutic antibody. "Inhibiting the growth of tumour cells expressing CD47" or "growth inhibition" is where a measurable growth inhibition of cancer  
20 cells (expressing or overexpressing CD47) is achieved. Preferred growth inhibitory anti-SIRP $\alpha$  antibodies inhibit growth of CD47-expressing tumour cells by greater than 20%, preferably from about 20% to about 50%, and even more preferably, by greater than 50% (e.g., from about 50% to about 100%) as compared to the appropriate control, the control typically being tumour cells not treated with the antibody being tested. In one embodiment, growth inhibition can be  
25 measured at an antibody concentration of about 0.1 to 30 mg/ml or about 0.5 nM to 200 nM in cell culture, where the growth inhibition is determined 1-10 days after exposure of the tumour cells to the antibody. Growth inhibition of tumour cells *in vivo* can be determined in various ways such as for example is described in EP2474557B1. The antibody is growth inhibitory *in vivo* if administration of the anti-SIRP $\alpha$  antibody at about 1 mg/kg to about 100 mg/kg  
30 bodyweight results in reduction in tumour size or tumour cell proliferation within about 5 days to 3 months from the first administration of the antibody, preferably within about 5 to 30 days.

An antibody which "induces cell death" is one which causes a viable cell to become non-viable. The cell is one which expresses CD47. Cell death *in vitro* may be determined in the absence of complement and immune effector cells to distinguish cell death induced by ADCC.

Thus, the assay for cell death may be performed using heat inactivated serum (i.e., in the absence of complement) in the absence of immune effector cells. To determine whether the antibody is able to induce cell death, loss of membrane integrity as evaluated by uptake of propidium iodide (PI), trypan blue (see Moore *et al. Cytotechnology* 1995; 17, 1-11), or 7-AAD  
5 can be assessed relative to untreated cells or loss of cell viability can be evaluated (tetrazolium reduction, resazurin reduction, protease markers, and ATP detection).

The expression "therapeutically effective amount" means an amount effective in treating cancer, as previously defined; said amount can be an amount sufficient to effect a desired response, or to ameliorate a symptom or sign, e.g., of metastasis or primary tumour progression,  
10 size, or growth. A therapeutically effective amount for a particular subject may vary depending on factors such as the condition being treated, the overall health of the subject, the method, route, and dose of administration and the severity of side effects. Response evaluation criteria have been described (RECIST; Eisenhauer *et al. European Journal of Cancer* 2009; 45, 228-247; Schwartz *et al. European Journal of Cancer* 2016; 62, 138-145; Cheson *et al. Journal of*  
15 *Clinical Oncology* 2003; 21(24), 4642-4649; Moghbel *et al. Journal of Nuclear Medicine* 2016, 57(6), 928-935; references included by reference in their entirety). Preferably, the effect will result in tumour stasis (i.e. no reduction but a *status quo*), a reduction in the number of lesions or a reduction in tumour size of at least about 10%, preferably at least 20%, 30%, 50%, 70%, or even 90% or more as compared to the baseline tumour size, preferably a reduction of at least  
20 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% in the sum of diameters of target lesions, taking as a reference the baseline sum diameters. When in combination, a therapeutically effective amount is in ratio to a combination of components and the effect is not limited to individual components alone. A therapeutically effective amount will modulate the symptoms preferably by at least about 10%; preferably by at least about 20%;  
25 preferably at least about 30%; or more preferably at least about 50%. Alternatively, modulation of migration will mean that the migration or trafficking of various cell types is affected. Such will result in, e.g., statistically significant and quantifiable, changes in the numbers of cells being affected. This may be a decrease in the numbers of target cells being attracted within a time period or target area. Rate of primary tumour progression, size, dissemination or growth  
30 may also be monitored.

In a preferred embodiment, the present invention relates to an anti-SIRP $\alpha$  antibody or antigen-binding fragment thereof or pharmaceutical composition as described hereinabove for use in the treatment of a CD47 expressing disease, condition or indication, in particular a cancer, more in particular a human solid tumour or haematological malignancy, in combination with

the use of a therapeutic antibody and further in combination with one or more other anti-cancer therapies. Suitable other anti-cancer therapies include, but are not limited to surgery, chemotherapy, radiation therapy, hormonal therapy, and small-molecule targeted therapy, such as for example angiogenesis inhibitors. The anti-SIRP $\alpha$  antibody or antigen-binding fragment thereof 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 or antigen-binding fragment thereof 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 antigen-binding fragment thereof or pharmaceutical composition as described hereinabove for use in the treatment of a CD47 expressing disease, condition or indication, in combination with the use of one or more further anti-cancer therapeutic compounds. An “anti-cancer therapeutic compound” as used herein is intended not to include a therapeutic antibody. A therapeutic antibody is defined hereinabove. Thus, the anti-SIRP $\alpha$  antibody or antigen-binding fragment thereof or pharmaceutical composition as described hereinabove, preferably in combination with a therapeutic antibody as defined above, may be for use in the treatment of human solid tumours and haematological malignancies before, after or simultaneously with the use of one or more other anti-cancer therapeutic compounds.

Suitable anti-cancer therapeutic compounds include a cytotoxic agent, i.e. a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include chemotherapeutic agents, i.e., chemical compounds useful in the treatment of cancer, radiation therapeutics, such as radioactive isotopes, hormonal therapeutics, targeted therapeutics and immunotherapeutic agents. Suitable chemotherapeutic agents include alkylating agents, such as nitrogen mustards, nitrosoureas, tetrazines and aziridines; anti-metabolites, such as anti-folates, fluoropyrimidines, deoxynucleoside analogues and thiopurines; anti-microtubule agents, such as vinca alkaloids and taxanes; topoisomerase I and II inhibitors; and cytotoxic antibiotics, such as anthracyclines and bleomycins. For example, the chemotherapy regimen may be selected from the group consisting of CHOP (cyclophosphamide, doxorubicin (also called hydroxyl daunorubicin), vincristine (also called oncovin) and prednisone), ICE (idarubicin, cytarabine and etoposide), mitoxantrone, cytarabine, DVP (daunorubicin, vincristine and prednisone), ATRA (all-trans retinoic acid), idarubicin, Hoelzer chemotherapy regimen, ABVD (bleomycin, dacarbazine, doxorubicin and

vincristine), CEOP (cyclophosphamide, epirubicin, vincristine and prednisolone), 2-CdA (2-chlorodeoxyadenosine), FLAG & IDA (fludarabine, cytarabine, filgrastim and idarubicin) (with or without subsequent G-CSF (granulocyte-colony stimulating factor) or GM-CSF treatment), VAD (vincristine, doxorubicin and dexamethasone), M & P (melphalan and prednisone), C (cyclophosphamide)-Weekly, ABCM (adriamycin, bleomycin, cyclophosphamide and mitomycin-C), MOPP (mechlorethamine, vincristine, prednisone and procarbazine) and DHAP (dexamethasone, cytarabine and cisplatin). A preferred chemotherapeutic regimen is CHOP. Suitable radiation therapeutics include radioisotopes, 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 synthesis, such as aromatase inhibitors and GnRH analogues; and hormone receptor antagonists, such as selective oestrogen receptor modulators and antiandrogens. A targeted therapeutic as used herein is a therapeutic that interferes with specific proteins involved in tumourigenesis and proliferation and may be a small molecule drug; or a peptide or peptide derivative. Examples of targeted small molecule drugs include mTOR inhibitors, such as everolimus, temsirolimus and rapamycin; kinase inhibitors, such as imatinib, dasatinib and nilotinib; VEGF inhibitors, such as sorafenib and regorafenib; and EGFR/HER2 inhibitors such as gefitinib, lapatinib and erlotinib. Examples of peptide or peptide derivative targeted therapeutics include proteasome inhibitors, such as bortezomib and carfilzomib. Immunotherapeutic agents include agents that induce, enhance or suppress an immune response, such as cytokines (IL-2 and IFN- $\alpha$ ); immunomodulatory imide drugs, such as thalidomide, lenalidomide and pomalidomide; therapeutic cancer vaccines, such as talimogene laherparepvec; cell based immunotherapeutic agents, such as dendritic cell vaccines, adoptive T-cells and chimeric antigen receptor-modified T-cells); or immunotoxins, such as moxetumomab pasudotox.

Any of the above mentioned therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals, preferably primates, including for example non-human primates, and most preferably, humans.

In this document and in its claims, the verb “to comprise” and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, reference to an element by the indefinite article “a” or “an” does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article “a” or “an” thus usually means “at least one”.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

The following Examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

5

## EXAMPLES

### 1. Transient expression of antibodies

#### a) Preparation of cDNA constructs and expression vectors

The heavy chain variable region (HCVR) amino acid sequences of the antibodies were each joined at the N-terminus to a leader sequence (SEQ ID NO:28 for antibodies 1-13), and at the C-terminus to the constant domain of a human IgG<sub>1</sub> HC LALA according to SEQ ID NO:25 (*in silico*). The HCVR amino acid sequences of antibodies 12C4, 12C4-LALA, 29AM4-5-LALA or KWAR23-LALA, were each joined at the N-terminus to a HAVT20 leader sequence (SEQ ID NO:27) and at the C-terminus to the constant domain of a human IgG<sub>1</sub> HC LALA according to SEQ ID NO:25 or a wild-type human IgG<sub>1</sub> HC (SEQ ID NO:24). KWAR23 has the standard adalimumab constant domain of the heavy chain, but lacks the LALA mutation. HEFLB heavy chain is an IgG4 and was used as disclosed SEQ ID NO:42 of WO 2017/178653. The resulting amino acid sequences were back-translated into a cDNA sequence codon-optimized for expression in human cells (*Homo sapiens*). Similarly, the cDNA sequence for the LC of the construct (light chain variable region; LCVR) was obtained by joining the sequences of a leader sequence (SEQ ID NO:29 for antibodies 1-13, SEQ ID NO:27 for 12C4, 12C4-LALA, 29AM4-5-LALA, KWAR23, KWAR23-LALA and (humanized) HEFLB) to the LCVR of antibodies 1-13, 12C4, 12C4-LALA, 29AM4-5-LALA, KWAR23, KWAR23-LALA and (humanized) HEFLB at the N-terminus and at the C-terminus to a human antibody  $\kappa$  light chain constant region (SEQ ID NO:26). The HCVR and LCVR sequences according to Table 1a-c were used. Anti-SIRP $\alpha$  antibody SE5A5 (mouse IgG<sub>1</sub> $\kappa$ ) was obtained from Biolegend (San Diego, USA; Purified anti-human CD172a/b (SIRP $\alpha$ / $\beta$  antibody)). The cDNA constructs and expression vectors for the comparative anti-SIRP $\alpha$  antibodies provided in Table 1c and the isotype controls provided in Table 1d were made similarly.

30

Table 1a presents (i) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 amino acid sequences (SEQ ID NOs) that are comprised in the heavy chain and the light chain in each of the humanized anti-SIRPα antibodies 1-13, and (ii) the amino acid sequences of each of the HCVR and LCVR including the CDRs of humanized anti-SIRPα antibodies 1-13.

| Antibody | HCVR         | HCDR1      | HCDR2 | HCDR3 | LCVR        | LCDR1      | LCDR2 | LCDR3 |
|----------|--------------|------------|-------|-------|-------------|------------|-------|-------|
|          |              | SEQ ID NO: |       |       |             | SEQ ID NO: |       |       |
| 1        | SEQ ID NO:1  | 30         | 31    | 32    | SEQ ID NO:2 | 33         | 34    | 35    |
| 2        | SEQ ID NO:3  | 30         | 31    | 32    | SEQ ID NO:2 | 33         | 34    | 35    |
| 3        | SEQ ID NO:4  | 30         | 31    | 32    | SEQ ID NO:2 | 33         | 34    | 35    |
| 4        | SEQ ID NO:5  | 30         | 31    | 32    | SEQ ID NO:2 | 33         | 34    | 35    |
| 5        | SEQ ID NO:6  | 36         | 37    | 38    | SEQ ID NO:7 | 39         | 40    | 46    |
| 6        | SEQ ID NO:8  | 36         | 44    | 45    | SEQ ID NO:9 | 39         | 40    | 41    |
| 7        | SEQ ID NO:10 | 36         | 37    | 38    | SEQ ID NO:9 | 39         | 40    | 41    |
| 8        | SEQ ID NO:6  | 36         | 37    | 38    | SEQ ID NO:9 | 39         | 40    | 41    |
| 9        | SEQ ID NO:11 | 36         | 37    | 38    | SEQ ID NO:9 | 39         | 40    | 41    |
| 10       | SEQ ID NO:12 | 36         | 42    | 43    | SEQ ID NO:9 | 39         | 40    | 41    |
| 11       | SEQ ID NO:13 | 36         | 37    | 38    | SEQ ID NO:7 | 39         | 40    | 46    |
| 12       | SEQ ID NO:14 | 30         | 31    | 32    | SEQ ID NO:2 | 33         | 34    | 35    |
| 13       | SEQ ID NO:15 | 30         | 31    | 32    | SEQ ID NO:2 | 33         | 34    | 35    |

Table 1b presents the HC and LC of control antibodies.

| Reference antibody                             | HC SEQ ID NO: | LC SEQ ID NO: |
|------------------------------------------------|---------------|---------------|
| 12C4 (IgG <sub>1</sub> )                       | 16            | 17            |
| 29AM4-5-LALA (IgG <sub>1</sub> )               | 18            | 19            |
| 12C4-LALA (IgG <sub>1</sub> )                  | 20            | 21            |
| KWAR23-LALA (chimeric IgG <sub>1</sub> , LALA) | 22            | 23            |
| KWAR23 (mouse IgG <sub>2a</sub> )              | 47            | 48            |
| HEFLB (humanized IgG <sub>4</sub> )*           | 49            | 50            |

\*HEFLB is an IgG<sub>4</sub> antibody, thus the heavy chain variable domain is not joined to an IgG<sub>1</sub> backbone, but to an IgG<sub>4</sub> Fc sequence (WO 2017/178653).

5 Table 1c presents the amino acid sequences of the HC and LC of further comparative anti-SIRP $\alpha$  antibodies. These antibodies were selected from WO2018/190719, WO2018/057669, WO2019/023347, WO2018/107058 and WO01/40307.

| Reference antibody | HC SEQ ID NO: | LC SEQ ID NO: | Description                                              |
|--------------------|---------------|---------------|----------------------------------------------------------|
| 1H9                | 57            | 58            | 1H9 humanized IgG <sub>1</sub> -kappa, HC-N297A          |
| 40A-1              | 59            | 60            | 40AVH2VL4 humanized IgG <sub>1</sub> -kappa, HC-N297A    |
| 40A-2              | 61            | 62            | 40AVH2VL4 humanized IgG <sub>2</sub> -kappa, HC-A378S    |
| AB3-LALA           | 63            | 64            | AB3 chicken-human chimeric IgG <sub>1</sub> -kappa, LALA |

| Reference antibody | HC SEQ ID NO: | LC SEQ ID NO: | Description                                            |
|--------------------|---------------|---------------|--------------------------------------------------------|
| AB25-LALA          | 65            | 66            | AB25 humanized IgG <sub>1</sub> -kappa, LALA           |
| AB115-LALA         | 67            | 68            | AB115 human IgG <sub>1</sub> -kappa, LALA              |
| AB119-LALA         | 69            | 70            | AB119 human IgG <sub>1</sub> -kappa, LALA              |
| AB136-LALA         | 71            | 72            | AB136 human IgG <sub>1</sub> -kappa, LALA              |
| 3F9-LALA           | 73            | 74            | 3F9 mouse-human chimeric IgG <sub>1</sub> -kappa, LALA |
| 7H9-LALA           | 75            | 76            | 7H9 mouse-human chimeric IgG <sub>1</sub> -kappa, LALA |

All reference antibodies HC and LC sequences comprise as a leader sequence the HAVT20 leader sequence of SEQ ID NO: 27. The leader sequence is expressed and is needed for transport out of the cell, during which process it is cut off.

Table 1d presents the list of isotype control antibodies

| Reference antibody | Description                                 |
|--------------------|---------------------------------------------|
| iso 1 - LALA       | humanized IgG <sub>1</sub> -kappa, LALA     |
| iso2               | humanized IgG <sub>1</sub> -kappa, HC-N297A |
| iso3               | humanized IgG <sub>2</sub> -kappa, HC-A378S |
| iso4               | mouse IgG <sub>2a</sub> , wt                |
| iso5               | humanized IgG <sub>4</sub>                  |
| iso6               | humanized IgG <sub>1</sub>                  |

| Reference antibody | Description                                       |
|--------------------|---------------------------------------------------|
| iso7*              | Purified Mouse IgG <sub>1</sub> , κ, BioLegend    |
| iso8               | humanized IgG <sub>4</sub> -kappa, HC-S228P,L445P |

\* iso7 was obtained from BioLegend (cat#400102).

b) Vector construction and cloning strategy

For expression of the antibody chains a mammalian expression vector was used (pcDNA3.4; ThermoFisher), which contains a CMV: TKpA expression cassette. The final vectors containing either the HC or the LC expression cassette (CMV:HC: TKpA and  
5 CMV:LC- TKpA, respectively) were transferred to and expanded in *E. coli* NEB 5- $\alpha$  cells. Large-scale production of the final expression vectors for transfection was performed using Maxi- or Megaprep kits (Qiagen).

c) Transient expression in mammalian cells

Commercially available Expi293F cells (Thermo Fisher) were transfected with the  
10 expression vectors using the ExpiFectamine transfection agent according to the 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  
15 centrifugation at 4,000 g for 15 min and filtering the clarified harvest over PES bottle filters/ MF 75 filters (Nalgene). Antibodies were purified by affinity chromatography.

## 2. Antibody binding and specificity

### *Experimental*

20 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 (SEQ ID NOs:51 – 56) were captured on the surface of a chip suitable for biotinylated molecules (Sensor Chip CAP, GE Life Sciences) by injecting 5  $\mu$ g/ml of the SIRP antigen in running buffer (10 mM HEPES buffer at  
25 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  $\mu$ l/min after injection of a 20x diluted (in running buffer) biotin CAPture reagents (GE Life Sciences) for 60 sec at 10  $\mu$ l/min. Baseline stabilization was set at 1 min after which five increasing concentrations of an anti-SIRP antibody in running buffer were injected. For each step an association time of 150 sec was used,  
30 followed by a dissociation time of 600 sec after the highest concentration only, all at a flow rate of 30  $\mu$ l/min. Regeneration was performed with 6 M guanidine-HCl, 0.25 M NaOH solution (120 sec with flow rate of 30  $\mu$ l/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 (association rate [ $k_a$ ], dissociation rate [ $k_d$ ] and binding constant, also referred to as equilibrium dissociation constant or binding affinity [ $K_D$ ]) were calculated using BiaCore™ T200 evaluation software (v3.1).

Flow Cytometry assay: U937 cells (human monocytic cell line) 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 either one of 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 0.1% 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 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 (for human antibodies, AffiniPure F(ab')<sub>2</sub> fragment Goat-anti-human IgG-APC, 1:6,000 dilution, Jackson Immuno Research was added and for mouse antibodies, AffiniPure Fab fragment Goat Anti-Mouse IgG (H+L)-Alexa Fluor 488, 1:1,000 dilution, Jackson Immuno Research was added).. After 30 min at 4°C, cells were washed twice and resuspended in 150  $\mu$ l FACS buffer. Fluorescence intensities were determined by flow cytometry (BD FACSVerser, Franklin Lakes, NJ) and indicated as the median fluorescence intensity (MFI-Median) for U937 cells and the mean fluorescence intensity (MFI-Mean) for 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 top of the curve, when using a 4-parameter logistic fit.

## Results

SPR assay: The  $K_D$  (i.e. binding constant, also referred to as 'equilibrium dissociation constant' or 'binding affinity') values for binding to human SIRP $\alpha_1$  (huSIRP $\alpha_1$ ), human SIRP $\alpha_{BIT}$  (huSIRP $\alpha_{BIT}$ ), cynomolgus monkey SIRP $\alpha$  (cySIRP $\alpha$ ), human SIRP $\gamma$  (huSIRP $\gamma$ ), human SIRP $\beta_{1v1}$  (huSIRP $\beta_{1v1}$ ) and human SIRP $\beta_{1v2}$  (huSIRP $\beta_{1v2}$ ) of antibodies 1-13 and reference antibodies are summarized in Table 2. Antibodies 1-13 bind to both huSIRP $\alpha_{BIT}$  and huSIRP $\alpha_1$ , and do not bind to huSIRP $\gamma$ . Some of antibodies 1-13, for example antibody 6, occasionally show weak association with SIRP $\gamma$ , but with a very low response unit (RU), which appears to be irrelevant as shown in the cellular binding experiments in the Examples below. Humanized HEFLB recognizes only the huSIRP $\alpha_{BIT}$  variant and not huSIRP $\alpha_1$  and huSIRP $\gamma$

and cySIRP $\alpha$ . KWAR23, 29AM4-5, SE5A5 and 12C4 antibodies bind to all SIRP variants, including huSIRP $\gamma$ .

Table 2. Binding affinities ( $K_D$  in M) of anti-SIRP $\alpha$  antibodies to human SIRP $\alpha_1$ , human SIRP $\alpha_{BIT}$ , human SIRP $\gamma$ , human SIRP $\beta_{1v1}$ , human SIRP $\beta_{1v2}$  and cynomolgus monkey SIRP $\alpha$  measured by SPR

| Antibody     | huSIRP $\alpha_1$ | huSIRP $\alpha_{BIT}$ | cySIRP $\alpha$ | huSIRP $\gamma$ | huSIRP $\beta_{1v2}$ | huSIRP $\beta_{1v1}$ |
|--------------|-------------------|-----------------------|-----------------|-----------------|----------------------|----------------------|
| 12C4         | 9.72E-11          | < 1.0E-11             | < 1.0E-11       | < 1.0E-11       | < 1.0E-11            | 1.01E-10             |
| 29AM4-5-LALA | 2.29E-11          | < 1.0E-11             | 2.21E-11        | 4.45E-10        | 8.54E-11             | 6.01E-11             |
| 12C4-LALA    | 1.01E-10          | < 1.0E-11             | < 1.0E-11       | < 1.0E-11       | < 1.0E-11            | 1.13E-10             |
| KWAR23-LALA  | 1.25E-11          | < 1.0E-11             | < 1.0E-11       | < 1.0E-11       | < 1.0E-11            | 3.20E-11             |
| KWAR23       | 1.44E-11          | < 1.0E-11             | < 1.0E-11       | < 1.0E-11       | < 1.0E-11            | 3.56E-11             |
| HEFLB        | n.r.              | 1.28E-11              | n.r.            | 1.74E-08#       | 2.16E-10             | 1.61E-10             |
| SE5A5        | 1.54E-11          | 7.98E-10              | 1.80E-09        | 2.66E-09        | 2.87E-10             | 3.82E-11             |
| 1            | 1.49E-09          | < 1.0E-11             | 1.87E-09        | n.r.            | 2.42E-09             | n.r.                 |
| 2            | 1.34E-09          | < 1.0E-11             | 2.27E-09        | n.r.            | 2.54E-09             | n.r.                 |
| 3            | 9.08E-10          | < 1.0E-11             | 1.66E-09        | n.r.            | 1.92E-09             | n.r.                 |
| 4            | 1.38E-09          | < 1.0E-11             | 1.94E-09        | n.r.            | 2.34E-09             | n.r.                 |
| 5            | < 1.0E-11         | 1.42E-10              | 7.62E-08#       | n.r.            | 2.97E-09             | 5.93E-10             |
| 6            | < 1.0E-11         | < 1.0E-11             | 4.56E-09        | n.r.            | 2.01E-09             | 7.43E-11             |
| 7            | 1.71E-11          | 3.78E-10              | n.r.            | n.r.            | n.r.                 | 1.65E-09             |
| 8            | < 1.0E-11         | 1.52E-10              | 1.88E-09#       | n.r.            | 1.84E-09             | 6.61E-10             |
| 9            | 1.91E-11          | 3.65E-10              | n.r.            | n.r.            | n.r.                 | 1.44E-09             |
| 10           | 6.72E-10          | 5.10E-09#             | n.r.            | n.r.            | n.r.                 | 1.29E-08             |
| 11           | 3.49E-11          | 2.12E-10              | n.r.            | n.r.            | n.r.                 | 2.01E-09             |
| 12           | 1.38E-09          | < 1.0E-11             | 1.53E-09        | n.r.            | 1.94E-09             | n.r.                 |
| 13           | 1.41E-09          | < 1.0E-11             | 2.05E-09        | n.r.            | 2.78E-09             | n.r.                 |

- The  $K_D$  values of huSIRP $\alpha_1$  and huSIRP $\alpha_{BIT}$  were obtained from a concentration series 1,56-6.25-25-100-400 ng/ml. The  $K_D$  values of cySIRP $\alpha$ , huSIRP $\gamma$ , huSIRP $\beta_{1v1}$  and huSIRP $\beta_{1v2}$  were obtained from a concentration series 6.25-25-100-400-1600 ng/ml. n.r.: no response or below a 10 response unit (RU) cut-off of the calculated  $R_{max}$ . When  $< 1.0E-11$  M is given as  $K_D$  value the sample could not be accurately determined because the affinity is outside the instrument range or the calculated  $K_D$  was around  $1.0E-11$  M, but surface saturation was seen. A  $K_D$  value of  $< 1.0E-11$  M means high affinity. # means sub-optimal fit to a 1:1 Langmuir model was observed.

- 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 as  $EC_{50}$  values, i.e. the antibody concentration in  $\mu\text{g/ml}$  that gives a response half way between bottom and top of the curve, which are shown in Table 3. Antibodies 1-13 bind to huSIRP $\alpha_{BIT}$  (either transiently expressed in ExpiCHO-S cells or endogenously expressed in U937 cells) and huSIRP $\alpha_1$ . Antibodies 1-4, 6, 12, 13 bind to cySIRP $\alpha$  in the low  $\mu\text{g/ml}$  range. These antibodies also bind, with the same  $EC_{50}$  value range (low  $\mu\text{g/ml}$ ), to U937 cells endogenously expressing huSIRP $\alpha_{BIT}$ . Reference antibodies KWAR23, KWAR23huIgG $_1$ LALA, 12C4huIgG $_1$ LALA, 12ChuIgG $_1$ , 29AM4-5huIgG $_1$ LALA and HEFLB show similar binding to huSIRP $\alpha_{BIT}$  expressed on U937 cells. HEFLB does not bind to huSIRP $\alpha_1$  and cySIRP $\alpha$ .

- Table 3. Cellular binding of anti-SIRP $\alpha$  antibodies to U937 cells endogenously expressing human SIRP $\alpha_{BIT}$  and to ExpiCHO-S cells transiently transfected with either human SIRP $\alpha_1$ , human SIRP $\alpha_{BIT}$  or cynomolgus monkey SIRP $\alpha$

| Antibody | U937 cells<br>(huSIRP $\alpha_{BIT}$ )<br>$EC_{50}$ ( $\mu\text{g/ml}$ ) | ExpiCHO-S<br>(huSIRP $\alpha_1$ )<br>$EC_{50}$ ( $\mu\text{g/ml}$ ) | ExpiCHO-S<br>(huSIRP $\alpha_{BIT}$ )<br>$EC_{50}$ ( $\mu\text{g/ml}$ ) | ExpiCHO-S<br>(cySIRP $\alpha$ )<br>$EC_{50}$ ( $\mu\text{g/ml}$ ) |
|----------|--------------------------------------------------------------------------|---------------------------------------------------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------|
| 1        | 0.09                                                                     | 0.28                                                                | 0.30                                                                    | 0.12                                                              |
| 2        | 0.10                                                                     | 0.32                                                                | 0.31                                                                    | 0.12                                                              |
| 3        | 0.12                                                                     | 0.40                                                                | 0.28                                                                    | 0.15                                                              |
| 4        | 0.10                                                                     | 0.38                                                                | 0.36                                                                    | 0.14                                                              |
| 5        | 1.60                                                                     | 0.22                                                                | 0.12                                                                    | 1.91                                                              |
| 6        | 0.14                                                                     | 0.24                                                                | 0.18                                                                    | 0.13                                                              |
| 7        | 3.55                                                                     | 0.20                                                                | 0.20                                                                    | 5.35                                                              |
| 8        | 1.04                                                                     | 0.17                                                                | 0.14                                                                    | 2.44                                                              |
| 9        | 2.78                                                                     | 0.19                                                                | 0.11                                                                    | >10                                                               |

| Antibody         | U937 cells<br>(huSIRP $\alpha$ <sub>BIT</sub> )<br>EC <sub>50</sub> (μg/ml) | ExpiCHO-S<br>(huSIRP $\alpha$ <sub>1</sub> )<br>EC <sub>50</sub> (μg/ml) | ExpiCHO-S<br>(huSIRP $\alpha$ <sub>BIT</sub> )<br>EC <sub>50</sub> (μg/ml) | ExpiCHO-S<br>(cySIRP $\alpha$ )<br>EC <sub>50</sub> (μg/ml) |
|------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------|-------------------------------------------------------------|
| 10               | 7.11                                                                        | 0.10                                                                     | 0.30                                                                       | >10                                                         |
| 11               | 3.73                                                                        | 0.11                                                                     | 0.08                                                                       | 6.30                                                        |
| 12               | 0.08                                                                        | 0.27                                                                     | 0.26                                                                       | 0.23                                                        |
| 13               | 0.08                                                                        | 0.27                                                                     | 0.28                                                                       | 0.11                                                        |
| 12C4             | 0.07                                                                        | 0.12                                                                     | 0.36                                                                       | 0.16                                                        |
| 29AM4-5-<br>LALA | 0.17                                                                        | 0.09                                                                     | 0.12                                                                       | 0.13                                                        |
| 12C4-LALA        | 0.04                                                                        | 0.11                                                                     | 0.17                                                                       | 0.22                                                        |
| KWAR23-<br>LALA  | 0.07                                                                        | 0.19                                                                     | 0.19                                                                       | 0.27                                                        |
| KWAR23           | 0.14                                                                        | 0.08                                                                     | 0.10                                                                       | 0.08                                                        |
| HEFLB            | 0.25                                                                        | > 30                                                                     | 0.15                                                                       | > 30                                                        |
| SE5A5            | 1.48                                                                        | 0.09                                                                     | 0.62                                                                       | 0.05                                                        |

### 3. Human SIRP $\gamma$ binding - T-cells FACS staining

#### *Experimental*

Flow Cytometry assay: Peripheral mononuclear cells (PBMC) were isolated from fresh  
5 blood of healthy individuals using Percoll gradient. Cells were washed in HEPES+ buffer (132 mM NaCl, 6 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, 1.2 mM potassium phosphate, 20 mM HEPES, 5.5 mM glucose and 0.5% (w/v) human serum albumin, pH 7.4) and resuspended in a concentration of 1x10<sup>6</sup>/ml in FACS buffer (PBS+human albumin 1% buffer w/v, Human Albumin 200 g/ml, Sanquin Plasma Products B.V., Amsterdam, Netherlands), spun down and  
10 resuspended in PBS+20% normal goat-serum (NGS). Cells (200,000 cells/well in a 96-well plate) were subsequently incubated in the presence of tested antibodies or control conditions with secondary antibodies only (secondary goat anti-human IgG Alexa 633 F(ab')<sub>2</sub> fragment, dilution 1:1000, Jackson Immuno Research, and secondary goat anti-mouse IgG Alexa 633 F(ab')<sub>2</sub> fragment, dilution 1:250, Invitrogen) for 30min on ice. After that, cells were washed  
15 with FACS buffer and resuspended in a mixture of anti-human CD3 FITC antibody (dilution 1:100, Invitrogen) with respective secondary antibody (either anti-human or anti-mouse) and

incubated for 30 min in the dark on ice. Afterwards the cells were washed with FACS buffer and resuspended in 150  $\mu$ l of FACS buffer and fluorescence intensities were determined by flow cytometry (LSRII HTS or LSRFortessa, BD Biosciences, CA, USA) and indicated as the median fluorescence intensity (MFI-Median) and percentage of positive cells.

5

### *Results*

Binding of antibodies to SIRP $\gamma$  expressing CD3<sup>+</sup> T-cells is shown in Figure 1 (mean fluorescence intensity (Figure 1a); percentage of positive cells (Figure 1b)). All reference antibodies, except for humanized HEFLB showed binding to SIRP $\gamma$ . Antibodies 1-13 did not  
10 show binding to human CD3<sup>+</sup> T-cells, confirming no binding of SIRP $\gamma$  in a cell-based environment.

## **4. Characterization of comparative anti-SIRP $\alpha$ antibodies**

### *Experimental*

Flow Cytometry assay: U937 cells (human monocytic cell line) endogenously expressing human SIRP $\alpha_{\text{BIT}}$  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 transiently expressing either human SIRP $\alpha_1$  or SIRP $\alpha_{\text{BIT}}$  antigen (100,000 cells/well in a 96-well plate) were washed twice with ice-cold FACS buffer (1x PBS (LONZA) containing 0.1% v/w BSA  
20 (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 antibody (50  $\mu$ l/well) diluted in ice-cold FACS buffer. After an incubation time of 30 min at 4°C, the cells were washed twice with ice-cold FACS buffer. Then, 50  $\mu$ l/well secondary mAb was added (AffiniPure™ F(ab')<sub>2</sub> fragment Goat-anti-human IgG-APC, 1:6,000 dilution, Jackson Immuno Research). After 30 min at 4°C,  
25 cells were washed twice and resuspended in 150  $\mu$ l FACS buffer. Fluorescence intensities were determined by flow cytometry with the FACSVerse (BD Biosciences) and indicated as the median fluorescence intensity (MFI-Median) for U937 cells and the mean fluorescence intensity (MFI-Mean) for ExpiCHO-S cells. Curves were fitted by nonlinear regression with a variable slope (four parameters) in GraphPad Prism (version 7.02 for Windows, GraphPad, San  
30 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 top of the curve, when using a 4-parameter logistic fit.

Surface Plasmon Resonance (SPR) assay: Affinity analysis was performed by single cycle kinetics analysis on a BiaCore™ T200 instrument (GE life Sciences) at 25°C. AVI-tagged

biotinylated SIRP antigens were captured using the Biotin CAPture kit (GE life Sciences). The streptavidin surface was prepared by injection of biotin capture reagent. Subsequently biotinylated SIRP variants were injected in running buffer (10mM HEPES buffer at pH 7.4 with 150 mM NaCl, 3 mM EDTA and 0.005% v/v Surfactant P20) to a capture level of approximately 40 – 50 response units (RU). After a 1 min baseline stabilization, five increasing concentrations of the anti-SIRP $\alpha$  antibodies were injected with an association time of 150 sec. The dissociation was observed for 600 sec, both at a flow rate of 30  $\mu$ l/min. The concentration range was chosen around the expected  $K_D$ . Regeneration was performed according to the manufacturer's protocol with 6M guanidine-HCl, 0.25M NaOH solution. Double reference subtraction was performed on the obtained sensorgrams using a (biotin capture reagent bound) reference flow channel and a 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). huSIRP $\beta_{1v1}$  and huSIRP $\beta_{1v2}$  were tested in their monomeric form. Estimated  $K_D$ s are within the tested concentration range. When  $< 1.0E^{-11}$  is the reported  $K_D$  value, the affinity could not accurately be determined because the kinetic parameters are outside the instruments specifications.

### Results

Flow cytometry assay: A comparison of SIRP $\alpha$  antibodies for binding to huSIRP $\alpha_1$  and huSIRP $\alpha_{BIT}$  expressed on cells was determined by flow cytometry. The binding is indicated as an  $EC_{50}$  value in Table 4. All antibodies tested here show binding to huSIRP $\alpha_{BIT}$  (either transiently expressed in Expi-CHO-S cells or endogenously expressed in U937 cells) and huSIRP $\alpha_1$ . While most antibodies display an  $EC_{50}$  value in the low  $\mu$ g/ml range, AB115-LALA, 3F9-LALA and 7H9-LALA show an  $EC_{50}$  value that is greater than 1  $\mu$ g/ml for binding to U937 cells. In addition, 3F9-LALA binds to huSIRP $\alpha_1$  and huSIRP $\alpha_{BIT}$  expressed on ExpiCHO-S cells with an  $EC_{50}$  value greater than 1  $\mu$ g/ml. Of note, corresponding isotype controls do not show binding to any of these cells.

Table 4. Cellular binding of anti-SIRP $\alpha$  antibodies to U937 cells endogenously expressing human SIRP $\alpha_{BIT}$  and to ExpiCHO-S cells transiently transfected with either human SIRP $\alpha_1$  or human SIRP $\alpha_{BIT}$ .

| Antibody    | U937 cells<br>(huSIRP $\alpha_{BIT}$ )<br>EC <sub>50</sub> ( $\mu$ g/ml) | ExpiCHO-S<br>(huSIRP $\alpha_1$ ) EC <sub>50</sub><br>( $\mu$ g/ml) | ExpiCHO-S<br>(huSIRP $\alpha_{BIT}$ )<br>EC <sub>50</sub> ( $\mu$ g/ml) |
|-------------|--------------------------------------------------------------------------|---------------------------------------------------------------------|-------------------------------------------------------------------------|
| 6           | 0.09                                                                     | 0.06                                                                | 0.01                                                                    |
| KWAR23-LALA | 0.07                                                                     | 0.14                                                                | 0.16                                                                    |
| 1H9         | 0.05                                                                     | 0.04                                                                | 0.08                                                                    |
| 40A-1       | 0.23                                                                     | 0.09                                                                | 0.16                                                                    |
| 40A-2       | 0.24                                                                     | 0.14                                                                | 0.12                                                                    |
| AB3-LALA    | 0.06                                                                     | 0.09                                                                | 0.13                                                                    |
| AB25-LALA   | 0.08                                                                     | 0.18                                                                | 0.10                                                                    |
| AB115-LALA  | 1.43                                                                     | 0.37                                                                | 0.48                                                                    |
| AB119-LALA  | 0.04                                                                     | 0.10                                                                | 0.07                                                                    |
| AB136-LALA  | 0.39                                                                     | 0.12                                                                | 0.18                                                                    |
| 3F9-LALA    | 3.24                                                                     | 1.49                                                                | 1.88                                                                    |
| 7H9-LALA    | 1.08                                                                     | 0.49                                                                | 0.52                                                                    |

- 5 SPR assay: A comparison of SIRP $\alpha$  antibodies for selectivity to human SIRP $\beta$ -variants  $\beta_{1v1}$  and  $\beta_{1v2}$  was performed using SPR and results are summarized in Table 5. Except for 3F9-LALA, all antibodies recognized huSIRP $\beta_{1v1}$ . All tested antibodies bind huSIRP $\beta_{1v2}$ , with antibody 6 having the highest K<sub>D</sub> and thus the lowest affinity. Of note, corresponding isotype controls do not show binding to huSIRP $\beta_{1v1}$  and to huSIRP $\beta_{1v2}$ .

10

Table 5. Binding affinities (K<sub>D</sub> in M) of anti-SIRP $\alpha$  antibodies to human SIRP $\beta_{1v1}$  and human SIRP $\beta_{1v2}$  as measured by SPR.

| Antibody    | huSIRP $\beta_{1v1}$ | huSIRP $\beta_{1v2}$ |
|-------------|----------------------|----------------------|
| 6           | 1.48E-10             | 3.20E-09             |
| KWAR23-LALA | 1.83E-11             | 2.52E-11             |

| Antibody     | huSIRP $\beta$ <sub>1v1</sub> | huSIRP $\beta$ <sub>1v2</sub> |
|--------------|-------------------------------|-------------------------------|
| 1H9          | 2.08E-11                      | 2.40E-11                      |
| 40A-1        | 7.15E-09                      | 7.44E-11                      |
| 40A-2        | 9.70E-09                      | 1.88E-10                      |
| AB3-LALA     | 2.12E-11                      | <1E-11                        |
| AB25-LALA    | <1E-11                        | <1E-11                        |
| AB115-LALA   | <1E-11                        | <1E-11                        |
| AB119-LALA   | <1E-11                        | 1.28E-11                      |
| AB136-LALA   | 1.71E-10                      | 2.02E-10                      |
| 3F9-LALA     | no binding                    | 3.80E-10                      |
| 7H9-LALA     | 3.36E-10                      | 3.99E-10                      |
| KWAR23       | 1.47E-11                      | 2.74E-11                      |
| 12C4         | 1.06E-10                      | 4.90E-11                      |
| 29AM4-5-LALA | 1.05E-10                      | 1.53E-10                      |
| 12C4-LALA    | 1.11E-10                      | 5.11E-11                      |
| HEFLB        | 2.20E-10                      | 3.46E-10                      |
| SE5A5        | 6.31E-11                      | 3.58E-10                      |

## 5. Binding of anti-SIRP $\alpha$ antibodies to primary cells: granulocytes, monocytes and T-cells

### *Experimental*

- 5 Flow Cytometry assay, whole blood staining: Heparinized whole blood samples were obtained from healthy donors (Sanquin blood bank Nijmegen, the Netherlands) and were stored overnight at room temperature. Whole blood samples were lysed with 1X BD FACSTM Lysing Solution (349202, BD Biosciences)) for 15 min at room temperature and washed with FACS buffer (PBS containing 0,1% BSA and 2 mM EDTA). 1.5x10<sup>5</sup> cells per well were stained with
- 10 50  $\mu$ l per well anti-SIRP $\alpha$  antibodies (concentration range starting from 10  $\mu$ g/ml or 90  $\mu$ g/ml with a 3.16x dilution) in 96-well microtiter plates (353910, Falcon) for 30 min at 4°C. After washing with FACS buffer, cells were incubated with a cocktail of 1:800 diluted anti-human CD3-PB clone UCHT1 (558117, BD Biosciences), 1:800 diluted anti-human CD14-FITC clone

MφP9 (345784, BD Biosciences) and 1:6000 diluted APC-labelled goat anti-human IgG F(ab')<sub>2</sub> secondary antibody (109-136-098, Jackson ImmunoResearch) in FACS buffer for 30 min at 4°C. After washing with FACS buffer, cells were mixed by vortexing to avoid cell aggregation and incubated with 50 µl per well BD Cytofix™ Fixation Buffer (4,2% PFA) (554655, BD Biosciences) for 15 min at room temperature and washed prior to analysis. Samples were collected with FACSVerse (BD Biosciences) and analysed in FlowJo Software (BD Biosciences). Granulocytes were gated based on FSC-A/SSC-A, followed by CD14<sup>-</sup> gating. T-cells and monocytes were first gated based on FSC-A/SSC-A. T-cells were then identified as CD3<sup>+</sup>CD14<sup>-</sup> cells and monocytes as CD14<sup>+</sup>CD3<sup>-</sup> cells.

10

Flow Cytometry assay, binding to isolated T-cells: T-cells were isolated by negative selection (11344D Dynabeads Untouched Human T Cell Kit, ThermoFisher Scientific) from peripheral blood mononuclear cells (PBMC) of healthy individuals (Sanquin blood bank Nijmegen, the Netherlands). Cells were washed in HEPES+ buffer (132 mM NaCl, 6 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, 1.2 mM potassium phosphate, 20 mM HEPES, 5.5 mM glucose and 0.5% (w/v) human serum albumin, pH 7.4) and resuspended in a concentration of 1x10<sup>6</sup>/ml in isolation buffer (PBS+human albumin 1% buffer w/v, Human Albumin 200 g/ml, Sanquin Plasma Products B.V., Amsterdam, Netherlands), spun down and resuspended in FACS buffer (1x PBS (LONZA) containing 0.1% v/w BSA (Sigma-Aldrich, St. Louis, MO) and 0.02% v/w NaN<sub>3</sub> (Sigma-Aldrich). Cells (100,000 cells/well in a 96-well plate) were washed with ice-cold FACS buffer followed by the addition of a concentration range of each primary mAb (50 µl/well) diluted in ice-cold FACS buffer. After an incubation time of 30 min at 4°C, the cells were washed two times with ice-cold FACS buffer; next 50 µl/well secondary antibody was added (for human antibodies, AffiniPure™ F(ab')<sub>2</sub> fragment Goat-anti-human IgG-APC, 1:6,000 dilution, Jackson Immuno Research; for mouse antibodies, AffiniPure™ Fab fragment Goat Anti-Mouse IgG (H+L)-Alexa Fluor 488, 1:1,000 dilution, Jackson Immuno Research). After 30 min at 4°C, cells were washed twice and resuspended in 150 µl FACS buffer. Fluorescence intensities were determined by flow cytometry with the FACSVerse (BD Biosciences) and indicated as the mean fluorescence intensity (MFI-Mean). Curves were fitted by nonlinear regression 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 µg/ml that gives a response half way between bottom and top of the curve, when using a 4-parameter logistic fit.

30

### Results

Flow Cytometry assay, whole blood staining: The binding of various SIRP $\alpha$  antibodies to primary cells was determined by flow cytometry. Dose-response curves for a representative healthy heterozygous SIRP $\alpha_1$ /SIRP $\alpha_{BIT}$  donor are shown in Figure 10a-d. It should be noted that the exact height of the response is not necessarily a characteristic of the SIRP $\alpha$  antibody as this can also depend on the secondary antibody. Instead, EC<sub>50</sub> values are independent of the detection antibody and should therefore be compared. A summary of the EC<sub>50</sub> values is depicted in Table 6. All antibodies show binding to granulocytes and monocytes of heterozygous SIRP $\alpha_{BIT}$ / SIRP $\alpha_1$  donors, though exact EC<sub>50</sub> values are varying. Except for HEFLB, all antibodies also show binding to granulocytes and CD14<sup>+</sup> monocytes of the homozygous SIRP $\alpha_1$  donor with fluctuating EC<sub>50</sub> values. These data are in line with the SPR and cellular data of Tables 2 and 3, where HEFLB also lacks binding to SIRP $\alpha_1$ . Circulating CD3<sup>+</sup> T-cells in the human blood do not express SIRP $\alpha$  or SIRP $\beta$  but only SIRP $\gamma$ , and therefore CD3<sup>+</sup> T-cell binding can be interpreted as SIRP $\gamma$  binding. While most antibodies show binding to CD3<sup>+</sup> T-cells, antibody 6, AB3-LALA, 3F9-LALA, 7H9-LALA and HEFLB do not show binding to CD3<sup>+</sup> T-cells. In this whole blood staining assay, AB136-LALA shows binding to T-cells at higher antibody concentrations. This appears to be in alignment with the disclosure in WO2018/057669 that Ab136 binds to SIRP $\gamma$ , but with a low KD.

Table 6. Cellular binding of anti-SIRP $\alpha$  antibodies to granulocytes, CD14<sup>+</sup> monocytes and CD3<sup>+</sup> T-cells in whole blood of healthy heterozygous SIRP $\alpha_1$ /SIRP $\alpha_{BIT}$  donors ( $\alpha_1/\alpha_{BIT}$ ) or a homozygous SIRP $\alpha_1$ /SIRP $\alpha_1$  donor ( $\alpha_1/\alpha_1$ ).

| Antibody    | Granulocytes             |                         |                     | CD14 <sup>+</sup> Monocytes |                         |                     | CD3 <sup>+</sup> T-cells (human ab) |                         |                     |
|-------------|--------------------------|-------------------------|---------------------|-----------------------------|-------------------------|---------------------|-------------------------------------|-------------------------|---------------------|
|             | EC <sub>50</sub> (μg/ml) |                         |                     | EC <sub>50</sub> (μg/ml)    |                         |                     | EC <sub>50</sub> (μg/ml)            |                         |                     |
|             | Donor #1                 | Donor #2                | Donor #3            | Donor #1                    | Donor #2                | Donor #3            | Donor #1                            | Donor #2                | Donor #3            |
|             | $\alpha_1/\alpha_{BIT}$  | $\alpha_1/\alpha_{BIT}$ | $\alpha_1/\alpha_1$ | $\alpha_1/\alpha_{BIT}$     | $\alpha_1/\alpha_{BIT}$ | $\alpha_1/\alpha_1$ | $\alpha_1/\alpha_{BIT}$             | $\alpha_1/\alpha_{BIT}$ | $\alpha_1/\alpha_1$ |
| 6           | 0.03                     | 0.03                    | 0.02                | 0.03                        | 0.03                    | 0.02                | >10                                 | >10                     | >10                 |
| KWAR23-LALA | 0.03                     | 0.04                    | 0.02                | 0.01                        | 0.02                    | 0.01                | 0.01                                | 0.01                    | 0.003               |
| 1H9         | 0.02                     | 0.04                    | 0.01                | 0.01                        | 0.03                    | 0.01                | *                                   | *                       | *                   |
| 40A-1       | 0.07                     | 0.06                    | 0.02                | 0.05                        | 0.05                    | 0.03                | 0.03                                | 0.03                    | 0.02                |
| AB3-LALA    | 0.01                     | 0.02                    | 0.01                | 0.01                        | 0.02                    | 0.01                | >10                                 | >10                     | >10                 |

|              | Granulocytes             |          |          | CD14+ Monocytes          |          |          | CD3+ T-cells (human ab)  |          |          |
|--------------|--------------------------|----------|----------|--------------------------|----------|----------|--------------------------|----------|----------|
|              | EC <sub>50</sub> (µg/ml) |          |          | EC <sub>50</sub> (µg/ml) |          |          | EC <sub>50</sub> (µg/ml) |          |          |
|              | Donor #1                 | Donor #2 | Donor #3 | Donor #1                 | Donor #2 | Donor #3 | Donor #1                 | Donor #2 | Donor #3 |
| Antibody     | α1/αBIT                  | α1/αBIT  | α1/α1    | α1/αBIT                  | α1/αBIT  | α1/α1    | α1/αBIT                  | α1/αBIT  | α1/α1    |
| AB25-LALA    | 0.02                     | 0.06     | 0.03     | 0.02                     | 0.06     | 0.03     | #                        | 0.06     | 0.03     |
| AB115-LALA   | 0.73                     | 0.35     | 0.18     | 0.61                     | 0.36     | 0.21     | 0.15                     | 0.09     | 0.10     |
| AB119-LALA   | 0.01                     | 0.01     | 0.005    | 0.01                     | 0.01     | 0.003    | 0.01                     | 0.01     | 0.005    |
| AB136-LALA   | *                        | *        | *        | *                        | *        | *        | *                        | *        | *        |
| 3F9-LALA     | 0.83                     | 0.51     | ~0.12^   | 0.48                     | 0.51     | ~ 0.11   | >10                      | >10      | >10      |
| 7H9-LALA     | 0.10                     | 0.08     | 0.04     | 0.10                     | 0.09     | 0.04     | >10                      | >10      | >10      |
| 29AM4-5-LALA | 0.03                     | 0.05     | 0.04     | 0.02                     | 0.03     | 0.02     | 0.07                     | 0.32     | 0.12     |
| 12C4-LALA    | 0.02                     | 0.03     | 0.10     | 0.01                     | 0.02     | 0.19     | *                        | *        | *        |
| HEFLB        | 0.02                     | 0.17     | >10      | 0.02                     | 0.15     | >10      | >10                      | >10      | >10      |

>10 = EC<sub>50</sub> > than 10 µg/ml

# = Excluded, resulted in an abnormal binding profile

^ = Dose-response curve is observed; however, the values are below isotype control

\* = Incomplete curve (saturation not reached)

~ = Ambiguous fitting

Flow Cytometry assay, binding to isolated T-cells: To confirm SIRP $\gamma$ -dependent T-cell binding of various antibodies using another approach, isolated primary T-cells (in absence of SIRP $\alpha$ - or SIRP $\beta$ -positive myeloid cells) were stained with the panel of antibodies. Results are shown in Figure 11. While most antibodies bind to T-cells, antibody 6, AB3-LALA, 3F9-LALA, 7H9-LALA, SE5A5 and HEFLB do not show binding to T-cells. Again, AB136-LALA shows binding at high antibody concentrations.

Thus, the Examples above show that the affinity of antibody 6 for huSIRP $\beta_{1v2}$  is lower than that of any of the comparative anti-SIRP $\alpha$  antibodies tested in the extensive panel listed above. Also, the affinity of non T-cell binding antibody 6 to SIRP $\beta_{1v1}$  was comparable to that of AB136-LALA, 7H9-LALA and HEFLB that also have low/no affinity for SIRP $\gamma$  and lower than that of AB3 and SE5A5 that also have low/no affinity for SIRP $\gamma$ .

In aggregate, antibody 6 has a high potency of binding to both SIRP $\alpha$ -alleles while simultaneously showing relatively low or absent binding to the other non-inhibitory SIRP-family members SIRP $\beta_{1v1}$ , SIRP $\beta_{1v2}$  and SIRP $\gamma$ .

## 5 6. CD47 blocking ability

### *Experimental*

CD47 blocking ability: To assess the ability of anti-SIRP $\alpha$  antibodies to block either SIRP $\alpha_1$  or SIRP $\alpha_{BIT}$  binding to CD47, SIRP $\alpha_1$  or SIRP $\alpha_{BIT}$  was pre-incubated with anti-SIRP $\alpha$  antibody and then dissociation from captured CD47 was tested. Briefly, AVI-tagged  
10 biotinylated CD47-Fc was captured using the Biotin CAPture kit (GE life Sciences). The streptavidin surface was prepared by injection of biotin capture reagent. Subsequently, biotinylated CD47-Fc was injected in running buffer (10 mM HEPES buffer at pH 7.4 with 150 mM NaCl, 3 mM EDTA and 0.005% v/v Surfactant P20) to a capture level of approximately 1000 RU. A mixture containing a fivefold molar excess of antibody and either 10  $\mu$ g/ml SIRP $\alpha_1$   
15 or 10  $\mu$ g/ml SIRP $\alpha_{BIT}$  was pre-incubated for 30 min at ambient temperature and injected over the CD47-Fc surface for 120 sec at 5  $\mu$ l/min. The dissociation was observed for 300 sec prior to regeneration with 6 M guanidine-HCl, 0.25 M NaOH (3:1), according to the manufacturer's instructions. Characterization of a blocking/non-blocking antibody was done by visual assessment after double reference subtraction.

20

### *Results*

CD47 blocking ability: The capacity of SIRP $\alpha$ -targeting antibodies to block CD47 binding was studied using SPR (Table 7). While most antibodies, including antibody 6, block the CD47-SIRP $\alpha_{BIT}$  and CD47-SIRP $\alpha_1$  interaction, AB3-LALA, AB136-LALA, 3F9-LALA  
25 and 7H9-LALA do not block CD47-SIRP $\alpha_{BIT}$  and CD47-SIRP $\alpha_1$  interaction and are thus non-blocking. In addition, HEFLB only blocks the interaction of CD47-SIRP $\alpha_{BIT}$ , but not that of CD47-SIRP $\alpha_1$ , consistent with the lack of recognition of SIRP $\alpha_1$  by HEFLB.

Table 7. Characterization of SIRP $\alpha$ -antibodies for blocking the CD47-SIRP $\alpha_{BIT}$  and CD47-SIRP $\alpha_1$  interactions.

| Antibody     | huSIRP $\alpha_1$ | huSIRP $\alpha_{BIT}$ |
|--------------|-------------------|-----------------------|
| 6            | yes               | yes                   |
| KWAR23-LALA  | yes               | yes                   |
| 1H9          | yes               | yes                   |
| 40A-1        | yes               | yes                   |
| 40A-2        | yes               | yes                   |
| AB3-LALA     | no                | no                    |
| AB25-LALA    | yes               | yes                   |
| AB115-LALA   | yes               | yes                   |
| AB119-LALA   | yes               | yes                   |
| AB136-LALA   | no                | no                    |
| 3F9-LALA     | no                | no                    |
| 7H9-LALA     | no                | no                    |
| KWAR23       | yes               | yes                   |
| 12C4         | yes               | yes                   |
| 29AM4-5-LALA | yes               | yes                   |
| 12C4-LALA    | yes               | yes                   |
| HEFLB        | no                | yes                   |
| SE5A5        | yes               | yes                   |

## 7. Ability to block SHP-1 recruitment

### 5 *Experimental*

SIRP $\alpha_{BIT}$  signalling was analysed using the PathHunter Enzyme Fragment Complementation technology from DiscoverX®. In this assay, CD47-deficient Jurkat cells are genetically engineered to over-express SIRP $\alpha_{BIT}^+$  tagged with ProLink (PK) and Enzyme Acceptor (EA) fused to the SH2 domain of the signalling protein SHP-1. When these Jurkat  
10 SIRP $\alpha_{BIT}$  signalling cells are incubated with cells that express CD47 (CD47 ligand cells), SHP-1 and SIRP $\alpha_{BIT}$  will interact resulting in the complementation of PK and EA. This creates an

active  $\beta$ -galactosidase enzyme that can cleave a substrate to generate a chemiluminescent signal. This system can be used to study the ability of SIRP $\alpha$ -targeting antibodies to antagonize SHP-1 recruitment to SIRP $\alpha$ . The Jurkat SIRP $\alpha_{BIT}$  signalling cells were incubated with a concentration range of anti-SIRP $\alpha$  antibody in combination with CD47 ligand cells. Jurkat E6.1  
5 cells were used as CD47 ligand cells and the assay was performed in 384 wells plate format. First, 12.5  $\mu$ l Jurkat SIRP $\alpha_{BIT}$  signalling cell suspension (0.8 million cells/ml) were added to each well followed by 2.5  $\mu$ l of a concentration range of anti-SIRP $\alpha$  antibody solution (11x concentrated). The assay was started by adding 12.5  $\mu$ l CD47 ligand cell suspension at the EC<sub>80</sub> (1.6 million Jurkat E6.1 cells/ml). Plates were incubated for 4 hours at 37°C and 5% CO<sub>2</sub>. After  
10 incubation, 2  $\mu$ l of 2x diluted reagents A (DiscoverX detection kit, in PBS containing 0.1% BSA) were added. Plates were incubated 30 min on a shaker (300 rpm), in the dark, at room temperature. Then, 10  $\mu$ l of 2x diluted reagents B (DiscoverX detection kit, in PBS containing 0.1% BSA) were added. Plates were incubated for 1 hour on a shaker (300 rpm) in the dark, at room temperature. Luminescence was measured at 0.1 sec/well integration time with the  
15 Envision® system (Perkin Elmer). All cell suspensions were prepared in cell plating medium (DiscoverX), the antibody dilutions in PBS containing 0.1% BSA (Sigma). Graphs were analysed in GraphPad Prism 8 software. The % of maximal signal was determined as follows: (relative luminescence units (RLU)/RLU of maximal stimulation (no antibody) \*100). Efficacy levels were calculated as followed: 100% - '% of maximum signal' of 3.3  $\mu$ g/ml compound  
20 value.

### Results

Activation of SIRP $\alpha$  leads to a well-characterized inhibitory signal cascade. Upon binding of CD47, the cytoplasmic domain immunoreceptor tyrosine-based inhibition motifs (ITIMs) of  
25 SIRP $\alpha$  become phosphorylated, leading to the recruitment and activation of Src homology region 2 domain-containing phosphatase-1 (SHP-1). SHP-1 mediates inhibitory signalling through protein dephosphorylation of specific substrates, including the activating Fc $\gamma$ Rs. This leads to dampening of the immune response. The ability of SIRP $\alpha$ -targeting antibodies to inhibit SIRP $\alpha$ -mediated signalling was studied using the CD47-deficient Jurkat SIRP $\alpha_{BIT}$  signalling  
30 cells. When these cells are incubated with CD47-containing Jurkat E6.1 cells, SHP-1 is recruited to SIRP $\alpha_{BIT}$  resulting in a chemiluminescent signal. Antibody 6 is able to antagonize this signal in a dose-dependent manner (Figure 12a). Other SIRP $\alpha$ -targeting antibodies were tested at a fixed dose (3.3  $\mu$ g/ml) for their ability to antagonize SIRP $\alpha_{BIT}$  signalling (Figure 12b). Except for SE5A5, the efficacy for inhibiting SIRP $\alpha_{BIT}$  signalling is similar for all CD47-

SIRP $\alpha$  blocking antibodies (depicted in white bars). The non-blocking antibodies AB3-LALA, AB136-LALA, 3F9-LALA and 7H9-LALA (depicted in black bars) display a lower or absent signal inhibitory efficacy compared to the blocking antibodies, and thus appear less or not capable of antagonizing SIRP $\alpha$ -mediated signalling.

- 5 In aggregate, antibody 6 blocks binding of CD47 to both SIRP $\alpha$ -alleles and in contrast to the non-blocking antibodies, this leads to a high inhibition of down-stream signalling.

## 8. Antibody Dependent Cellular Cytotoxicity (ADCC)

### *Experimental*

- 10 DELFI<sup>®</sup> cytotoxicity assay (non-radioactive assay): Neutrophils of donors heterozygous for SIRP $\alpha_1$  and SIRP $\alpha_{BIT}$  were isolated and cultured according to the method in Zhao *et al. PNAS* 2011, 108(45), 18342-18347. Freshly isolated neutrophils were cultured overnight with human G-CSF (10 ng/ml) and IFN $\gamma$  (50 ng/ml). Antibody Dependent Cellular Cytotoxicity (ADCC) was determined using the non-radioactive Europium TDA (EuTDA)
- 15 cytotoxicity assay (DELFI<sup>®</sup>, PerkinElmer). SKBR3 (human HER2-positive breast cancer cell line) cells were used as target cells and labelled with bis(acetoxymethyl)2,2':6',2''-terpyridine-6,6''-dicarboxylate (BATDA reagent, DELFI<sup>®</sup>) for 5 min at 37°C. After 2 washes with PBS, 5x10<sup>3</sup> target cells per well were incubated in IMDM culture medium supplemented with 10% (v/v) ultra-low IgG foetal bovine serum (FBS, Gibco) for 4 hours at 37°C and 5% CO<sub>2</sub> in a 96-
- 20 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 added to europium solution (DELFI<sup>®</sup>, PerkinElmer) and europium 2,2':6',2''-terpyridine-6,6''-dicarboxylic acid (EuTDA) fluorescence was determined using a spectrofluorometer (Envision, PerkinElmer). The percentage of cytotoxicity was calculated as [(experimental release -
- 25 spontaneous release) / (total release - spontaneous release)] x 100%. All conditions were measured in duplicate and/or triplicate.

### <sup>51</sup>Cr release assay (radioactive assay):

- Neutrophils of donors homozygous for either SIRP $\alpha_1$  or SIRP $\alpha_{BIT}$  were isolated according
- 30 to the method in Zhao *et al. PNAS* 2011, 108(45), 18342-18347. For all <sup>51</sup>Cr release assay experiments, except for those presented in Figure 2, freshly isolated neutrophils were cultured for 100 min with 10 ng/ml of human GM-CSF. Antibody Dependent Cellular Cytotoxicity (ADCC) was determined using the <sup>51</sup>Cr release assay. SKBR3 (human breast cancer cell line) cells were used as target cells and labelled with 100  $\mu$ Ci <sup>51</sup>Cr (Perkin-Elmer) for 90 min at 37°C.

After 2 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 for 4 hours at 37°C and 5% CO<sub>2</sub> 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 analysed for radioactivity in a gamma counter (Wallac). The percentage of cytotoxicity was calculated as [(experimental release - spontaneous release) / (total release - spontaneous release)] x 100%. All conditions were measured in duplicate and/or triplicate.

### Results

Humanization of 12C4 resulted in loss of ADCC, which was recovered by reduced effector function in the IgG<sub>1</sub> constant region:

Figure 2 shows the results of the ADCC assay as cytotoxicity in % as measured by a <sup>51</sup>Cr release assay. The % cytotoxicity measured on SKBR3 cells using neutrophils from homozygous SIRP $\alpha_{BIT}$  donors as effector cells and trastuzumab alone is less than the % cytotoxicity of trastuzumab in combination with the murine 12C4 antibody (mu12C4). Trastuzumab in combination with an antibody wherein 12C4 variable regions are grafted onto a human 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> 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 0.2 µg/ml 12C4huIgG<sub>1</sub> and trastuzumab.

Dose-dependent increase in trastuzumab mediated ADCC:

The percentage ADCC/cytotoxicity of trastuzumab (10 µg/ml) in the presence of antibody 1-13 – having a human IgG<sub>1</sub> constant region comprising the amino acid substitutions L234A and L235A (LALA) – or in the presence of reference antibody, at various concentrations (µg/ml; dose response curves) on SRBR3 HER2-positive breast cancer cells is shown in Figures 3-9. For antibodies 1-13 the ADCC dose-dependently increased in heterozygous SIRP $\alpha_1$ /SIRP $\alpha_{BIT}$  donors (Figures 4 and 5), whereas a dose-dependent decrease is seen for 12C4huIgG<sub>1</sub>, no clear effect is seen for humanized HEFLB, minimal effects are seen for KWAR23huIgG<sub>1</sub> and SE5A5 and a dose-dependent increase is seen for KWAR23-LALA reference antibody in heterozygous donors (Figure 3). Antibodies 7-13 and the

reference antibodies were also tested on homozygous SIRP $\alpha_1$  or SIRP $\alpha_{BIT}$  backgrounds (Figures 6-9). All antibodies appeared to show more variable results.

## 9. Immunogenicity

5 CD4<sup>+</sup> T-cell epitopes are important drivers of immunogenicity (anti-drug antibodies) *in vivo*. An *ex vivo* T-cell assay was used to detect T-cell responses against T-cell epitopes in anti-SIRP $\alpha$  antibody 6. Antibody 6 was sent for immunogenicity assessment using EpiScreen<sup>TM</sup> time course T-cell assay for their ability to induce CD4<sup>+</sup> T-cell responses at Abzena (Cambridge, UK). PBMC from a cohort of 50 healthy donors representing the European and  
10 North American population (based on HLA allotypes) were incubated with the test samples. T-cell responses were measured using proliferation assays (<sup>3</sup>H]-thymidine uptake) and cytokine secretion assays (IL-2 ELISpot). Antibody 6 was found not immunogenic in a combination of T-cell proliferation assay and IL-2 ELISpot.

## 15 10. Antibody Dependent Cellular Cytotoxicity (ADCC); comparative example

### *Experimental*

Human neutrophils of donors homozygous for either SIRP $\alpha_1$  or SIRP $\alpha_{BIT}$ , or heterozygous for both alleles were isolated according to the method in Zhao *et al. PNAS* 2011, 108(45), 18342-18347. Neutrophils were then stimulated with 10 ng/ml of granulocyte-monocyte colony stimulating factor (GM-CSF, Peprotech) for 30 min. Antibody Dependent  
20 Cell Cytotoxicity (ADCC) was determined using the <sup>51</sup>Cr release assay (PerkinElmer) according to the method in Zhao *et al. PNAS* 2011, 108(45), 18342-18347. Briefly, SKBR3 (human breast cancer cell line) or A431 (skin epidermoid carcinoma cell line) cells were used as target cells and labelled with 100  $\mu$ Ci <sup>51</sup>Cr (Perkin-Elmer) for 90 min at 37°C. After 2 washes  
25 with PBS, 5x10<sup>3</sup> target cells per well were opsonized with trastuzumab (10  $\mu$ g/ml final concentration for SKBR3), or cetuximab (5  $\mu$ g/ml final concentration for A431) and incubated in IMDM culture medium supplemented with 20% (v/v) low IgG foetal bovine serum (FBS) for 4 hours at 37°C and 5% CO<sub>2</sub> in a 96-well U-bottom plate together with neutrophils in an effector to target cell ratio of 50:1 in the presence of a dose-response range of the antibodies  
30 indicated in Figures 13-14. After the incubation, supernatant was harvested, transferred to LumaPlates (Perkin Elmer) and analysed for radioactivity in a MicroBeta counter (Perkin Elmer). The percentage of cytotoxicity was calculated as [(experimental release - spontaneous release) / (total release - spontaneous release)] x 100%. All conditions were measured in duplicate or triplicate.

*Results*

Antibody Dependent Cell Cytotoxicity (ADCC): The effect of anti-SIRP $\alpha$  antibodies was tested in ADCC experiments using GM-CSF activated primary human neutrophils as effector cells and different combinations of cancer-targeting therapeutic antibodies and tumour target cells (Figures 13a-b, 14a-b). The latter included HER2-expressing SKBR3 breast cancer cells in combination with trastuzumab (Figure 13) and EGFR-expressing A431 carcinoma cells in combination with cetuximab (Figure 14). The results demonstrate that antibody 6 was able to enhance neutrophil ADCC of both cancer-targeting antibody-target cancer cell combinations. Similar findings were obtained for several other anti-SIRP $\alpha$  antibodies, but not for e.g. 40A-1, 40A-2, 3F9-LALA, 7H9-LALA, 12C4, 29AM4-5-LALA, SE5A5.

In aggregate, antibody 6 is the only antibody with relatively low or absent affinity for the non-inhibitory SIRP-family members SIRP $\beta_{1v1}$ , SIRP $\beta_{1v2}$  and SIRP $\gamma$ , that in functional assays effectively inhibits down-stream signalling while simultaneously displaying enhanced ADCC in both SIRP $\alpha_{BIT}$  and SIRP $\alpha_1$  genotypes.

Sequence listings, antibodies 1-13 have underlined CDR1, CDR2 and CDR3 amino acid sequences in heavy chain (HC) and light chain (LC) variable region (VR) amino acid sequences (VR residues determined according to the method of Kabat; numbering of the sequences is sequentially, not according to the numbering of Kabat)

5

SEQ ID NO:1 (HCVR; mAb 1)

1 VQLVESGGRL GQPGTPLTSL CTVSGFSLSS YVMGWFRQAP GKGLEYIGII  
 51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SLRSEDTATY FCARVGPLGV  
 101 DYFNIWGPGT LVTVSS

10

SEQ ID NO:2 (LCVR; mAb 1, 2, 3, 4, 12, 13)

1 DIQMTQSPSS LSASVGDRVT ITCRASQSIN SYLAWYQQKP GKAPKLLIYS  
 51 ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQS WHYISRSYTF  
 101 GQGTEKVEIK

15

SEQ ID NO:3 (HCVR; mAb 2)

1 VQLVESGGRL VQPGTPLTSL CTVSGFSLSS YVMGWFRQAP GKGLEYIGII  
 51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SLRSEDTATY FCARVGPLGV  
 101 DYFNIWGPGT LVTVSS

20

SEQ ID NO:4 (HCVR; mAb 3)

1 VQLVESGGRL GQPGTSLTSL CTVSGFSLSS YVMGWFRQAP GKGLEYIGII  
 51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SPTTEDTATY FCARVGPLGV  
 101 DYFNIWGPGT LVTVSS

25

SEQ ID NO:5 (HCVR; mAb 4)

1 VQLVESGGRL GQPGTSLTSL CTVSGFSLSS YVMGWFRQAP GKGLEYIGII  
 51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SLRSEDTATY FCARVGPLGV  
 101 DYFNIWGPGT LVTVSS

30

SEQ ID NO:6 (HCVR; mAb 5, 8)

1 RQLVESGGGL VQPGGSLRLS CAASGFSLSS HGISWVRQAP GKGLEYIGTI  
 51 GTGVITYYAS WAKGRFTGSK TSSTAYLQMT SLRAEDTAVY YCARGSAWND  
 101 PFDYWGQGT LVTVSS

## SEQ ID NO:7 (LCVR; mAb 5, 11)

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

5

## SEQ ID NO:8 (HCVR; mAb 6)

1 RQLVESGGGL VQPGGSLRLS CTASGFSLS HGISWVRQAP GKGLEIYIGTI  
 51 GTGVITYFAS WAKGRFTGSK TSSTAYMELS SLRSED TAVY FCARGSAWND  
 101 PFDPWGQGTL VTVSS

10

## SEQ ID NO:9 (LCVR; mAb 6, 7, 8, 9, 10)

1 DIVMTQSPSS LSASVGDRVT ITCQASQSVY GNNDLAWYQQ KPGQAPKLLI  
 51 YLASTLATGV PSRFSGSGSG TDFTLTISL QPEDFATYYC LGGGDDEADN  
 101 TFGQGKVEI K

15

## SEQ ID NO:10 (HCVR; mAb 7)

1 RQLVESGGGL VQPGGSLRLS CAASGFSLS HGISWVRQAP GKGLEWVGTI  
 51 GTGVITYYAS WAKGRFTGSK TSSTAYLQMT SLRAED TAVY YCARSAWND  
 101 PFDYWGQGTL VTVSS

20

## SEQ ID NO:11 (HCVR; mAb 9)

1 RQLVESGGGL VQPGGSLRLS CAASGFSLS HGISWVRQAP GKGLEWVGTI  
 51 GTGVITYYAS WAKGRFTGSK TSSTAYLQMT SLRSED TAVY YCARSAWND  
 101 PFDYWGQGTL VTVSS

25

## SEQ ID NO:12 (HCVR; mAb 10)

1 RQLVESGGGL VQPGGSLRLS CAASGFSLS HGISWVRQAP GKGLEWVGTI  
 51 GTGGITYYAS WAKGRFTGSK TSSTAYMELS SLRAED TAVY YCARSAWND  
 101 PFDIWGQGTL VTVSS

30

## SEQ ID NO:13 (HCVR; mAb 11)

1 RQLVESGGGL VQPGGSLRLS CAASGFSLS HGISWVRQAP GKGLEWVGTI  
 51 GTGVITYYAS WAKGRFTGSK TSSTAYLQMT SLRAED TAVY YCARSAWND  
 101 PFDYWGQGTL VTVSS

## SEQ ID NO:14 (HCVR; mAb 12)

1 QSVESGGRL GQPGTPLTLS CTVSGFSLSS YVMGWFRQAP GKGL~~EYIGII~~  
 51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SLRSED~~TATY~~ FCARVGPLGV  
 101 DYFNIWGPGT LVTVSS

5

## SEQ ID NO:15 (HCVR; mAb 13)

1 VQLVESGGRL VQPGTPLTLS CTVSGFSLSS YVMGWFRQAP GKGL~~EYIGII~~  
 51 SSSGSPYYAS WVNGRFTISK TSTTMDLKMN SPTTED~~TATY~~ FCARVGPLGV  
 101 DYFNIWGPGT LVTVSS

10

## SEQ ID NO:16 (HC; 12C4)

1 EVKLEESGGG LMQPGGSMKL SCVASGFTFS NYWMNWVRQS PEKGLEWVAE  
 51 IRLKSN~~NYAT~~ HYAESVKGRF TISRDDSKSS VYLQMNNLRA EDTGIYYCIR  
 101 D~~YDYDAYFDY~~ WGQGTTLTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV  
 15 151 K~~DYFPEPVT~~ V SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ  
 201 TYICNVNHKP SNTKVDKKVE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK  
 251 PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY  
 301 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP  
 351 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTTP  
 20 401 VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG  
 451 K

## SEQ ID NO:17 (LC; 12C4)

1 DIVLTQSPAS LAVSLGQRAT ISCRASKSVS TSGYNYMYWY QQKPGQPPKL  
 25 51 LIYLASNLES GVPARFSGSG SGTDFTLNIH PVEEEDAATY YCQHS~~GELPY~~  
 101 TFGGGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV  
 151 QWKVDNALQS GNSQESVTEQ DSKDSTYSL S~~TLT~~LSKADY EKHKVYACEV  
 201 THQGLSSPVT KSFNRGEC

## 30 SEQ ID NO:18 (HC; 29AM4-5-LALA)

1 EVQLVESGGG LVQPGGSLRL SCAASGFNIS YYFIHWVRQA PGKGLEWVAS  
 51 VYSSFGY~~TTY~~ ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARFT  
 101 FPGLFDGFFG AYLGS~~LDYWG~~ QGTLVTVSSA STKGPSVFPL APSSKSTSGG  
 151 TAALGCLVKD YFPEPVT~~SW~~ NSGALTSGVH TFP~~AVLQSSG~~ LYSLS~~SVVTV~~

201 PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPEAAGGP  
 251 SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK  
 301 TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK  
 351 AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE  
 5 401 NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ  
 451 KSLSLSPGK

## SEQ ID NO:19 (LC; 29AM4-5-LALA)

1 DIQMTQSPSS LSASVGDRVT ITCRASQSVS SAVAWYQQKP GKAPKLLIYS  
 10 51 ASSLYSGVPS RFGSGRSGTD FTLTISSLQP EDFATYYCQQ AVNWWGALVT  
 101 FGQGTKVEIK RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ  
 151 WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLISKADYE KHKVYACEVT  
 201 HQGLSSPVTK SFNRGEC

## 15 SEQ ID NO:20 (HC; 12C4-LALA)

1 EVKLEESGGG LMQPGGSMKL SCVASGFTFS NYWMNWVRQS PEKGLEWVAE  
 51 IRLKSNYYAT HYAESVKGRF TISRDDSKSS VYLQMNNLRA EDTGIYYCIR  
 101 DYDYDAYFDY WGQGTTLTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV  
 151 KDYFPEPVTW SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ  
 20 201 TYICNVNHKP SNTKVDKKVE PKSCDKTHTC PPCAPEAAG GPSVFLFPPK  
 251 PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY  
 301 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP  
 351 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTTP  
 401 VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG  
 25 451 K

## SEQ ID NO:21 (LC; 12C4-LALA)

1 DIVLTQSPAS LAVSLGQRAT ISCRASKSVS TSGYNYMYWY QQKPGQPPKL  
 51 LIYLASNLES GVPARFSGSG SGTDFTLNIH PVEEEDAATY YCQHSSELGY  
 30 101 TFGGGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV  
 151 QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHVYACEV  
 201 THQGLSSPVT KSFNRGEC

## SEQ ID NO:22 (HC; KWAR23-LALA)

```

1    EVQLQQSGAE LVKPGASVKL SCTASGFNIK DYYIHWVQQR TEQGLEWIGR
51   IDPEDGETKY APKFQDKATI TADTSSNTAY LHLSSLTSED TAVYYCARWG
101  AYWGQGTLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV
5    151   TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH
201  KPSNTKVDKK VEPKSCDKTH TCPPCPAPEA AGGPSVFLFP PKPKDTLMIS
251  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
301  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
351  RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
10   401  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK

```

## SEQ ID NO:23 (LC; KWAR23-LALA)

```

1    QIVLTQSPAI MSASPGKVT LTCSASSSVS SSYLYWYQQK PGSSPKLWIY
51   STSNLASGVP ARFSGSGSGT SYSLTISME AEDAASYFCH QWSSYPRTFG
15   101  AGTKLELKRT VAAPSVFIFP PSDEQLKSGT ASVVCLLNNF YPREAKVQWK
151  VDNALQSGNS QESVTEQDSK DSTYLSSTL TLSKADYEKH KUYACEVTHQ
201  GLSSPVTKSF NRGEC

```

SEQ ID NO:24 (human IgG<sub>1</sub> antibody HC constant region)

```

20   1    ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
51   HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP
101  KSCDKHTTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
151  HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
201  EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC
25   251  LVKGFYPSDI AVEWESNGQP ENNYKTPPV LDSDGSFFLY SKLTVDKSRW
301  QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

```

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

```

30   1    ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
51   HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP
101  KSCDKHTTCP PCPAPEAAGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
151  HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
201  EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC

```

251 LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW  
 301 QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

SEQ ID NO:26 (human antibody LC  $\kappa$  constant region)

5 1 RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG  
 51 NSQESVTEQD SKDSTYSLSS TLTLKADYE KHKVYACEVT HQGLSSPVTK  
 101 SFNRGEC

10 SEQ ID NO:27 (HAVT20 leader sequence 12C4 / 12C4-LALA / 29AM4-5 LALA /  
 KWAR23 / KWAR23-LALA / HEFLB)

1 MACPGFLWAL VISTCLEFSM A

## SEQ ID NO:28 (leader sequence heavy chains mAb 1-13)

1 MGWTLVFLFL LSVTAGVHS  
 15

## SEQ ID NO:29 (leader sequence light chains mAb 1-13)

1 MVSSAQFLGL LLLCFQGTRC

## SEQ ID NO:30 (HCDR1; mAb 1, 2, 3, 4, 12 and 13)

20 1 SYVMG

## SEQ ID NO:31 (HCDR2; mAb 1, 2, 3, 4, 12 and 13)

1 IISSSGSPYY ASWVNG

## 25 SEQ ID NO:32 (HCDR3; mAb 1, 2, 3, 4, 12 and 13)

1 VGPLGVDFYFN I

## SEQ ID NO:33 (LCDR1; mAb 1, 2, 3, 4, 12 and 13)

1 RASQSINSYL A  
 30

## SEQ ID NO:34 (LCDR2; mAb 1, 2, 3, 4, 12 and 13)

1 SASFLYS

SEQ ID NO:35 (LCDR3; mAb 1, 2, 3, 4, 12 and 13)

1 QSWHYISRSY T

SEQ ID NO:36 (HCDR1; mAb 5-11)

5 1 SHGIS

SEQ ID NO:37 (HCDR2; mAb 5, 7, 8, 9, 11)

1 TIGTGVITYY ASWAKG

10 SEQ ID NO:38 (HCDR3; mAb 5, 7, 8, 9, 11)

1 GSAWNDPFDY

SEQ ID NO:39 (LCDR1; mAb 5-11)

1 QASQSVYGNN DLA

15

SEQ ID NO:40 (LCDR2; mAb 5-11)

1 LASTLAT

SEQ ID NO:41 (LCDR3; mAb 6-10)

20 1 LGGGDDEADN T

SEQ ID NO:42 (HCDR2; mAb 10)

1 TIGTGGITYY ASWAKG

25 SEQ ID NO:43 (HCDR3; mAb 10)

1 GSAWNDPFDI

SEQ ID NO:44 (HCDR2; mAb 6)

1 TIGTGVITYF ASWAKG

30

SEQ ID NO:45 (HCDR3; mAb 6)

1 GSAWNDPFDP

## SEQ ID NO:46 (LCDR3; mAb 5, 11)

1 LGGGDDEADN V

## SEQ ID NO:47 (HC; KWAR23)

5 1 EVQLQQSGAE LVKPGASVKL SCTASGFNIK DYYIHWVQQR TEQGLEWIGR  
 51 IDPEDGETKY APKFQDKATI TADTSSNTAY LHLSSLTSED TAVYYCARWG  
 101 AYWGQGTLLVT VSSAKTTAPS VYPLAPVCGD TTGSSVTLGC LVKGYFPEPV  
 151 TLTWNSGSLG SGVHTFPAVL QSDLYTLSSS VTVTSSTWPS QSITCNVAHP  
 201 ASSTKVDKKI EPRGPTIKPC PPCKCPAPNL LGGPSVFIFP PKIKDVLNIS  
 10 251 LSPIVTCVVV DVSEDDPDVQ ISWVNNVEV HTAQTQTHRE DYNSTLRVVS  
 301 ALPIQHQQDM SGKEFKCKVN NKDLPAPIER TISKPKGSVR APQVYVLPVP  
 351 EEEMTKKQVT LTCMVTDFMP EDIYVEWTNN GKTELNYKNT EPLVDSGDSY  
 401 FMYSKLRVEK KNWVERNSYS CSVVHEGLHN HHTTKSFSRT PGK

## 15 SEQ ID NO:48 (LC; KWAR23)

1 QIVLTQSPAI MSASPGEKVT LTCSASSSVS SSYLYWYQQK PGSSPKLWIY  
 51 STSNLASGVP ARFSGSGSGT SYSLTISSME AEDAASYFCH QWSSYPRTFG  
 101 AGTKLELKRA DAAPTVSIFP PSSEQLTSGG ASVVCFLNNF YPKDINVKWK  
 151 IDGSRQNGV LNSWTDQDSK DSTYSMSSTL TLTKEDEYERH NSYTCEATHK  
 20 201 TSTSPIVKSF NRNEC

## SEQ ID NO:49 (HC; HEFLB)

1 EVQLVQSGAE VKKPGESLRI SCKASGYSFT SYWVHWVRQM PGKGLEWMGN  
 51 IDPSDSDTHY SPSFQGHVTL SVDKSISTAY LQLSSLKASD TAMYYCVRGG  
 25 101 TGTLAYFAYW GQGTLLTVSS ASTKGPSVFP LAPCSRSTSE STAALGCLVK  
 151 DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT  
 201 YTCNVDPKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT  
 251 LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY  
 301 RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT  
 30 351 LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDL  
 401 DGSFFLYSRL TVDKSRWQEG NVFSCSVME ALHNHYTQKS LSLSPGK

## SEQ ID NO:50 (LC; HEFLB)

1 DVVMTQSPPLS LPVTLGQPAS ISCRSSQSLV HSYGNTYLYW FQQRPGQSPR

51 LLIYRVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCFQGTHVP  
 101 YTFGGGKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPPREK  
 151 VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE  
 201 VTHQGLSSPV TKSFNRGEC

5

SEQ ID NO:51 (human SIRP $\alpha_1$  extracellular domain 1-370, *Avi-FXa-Fc tag*)

MEPAGPAPGRLGPLLCLLLAASCAWSGVAGEEELQVIQPKSVSVAAGESAILHCTVTSLIP  
 VGPIQWFRGAGPARELIYNQKEGHFPRVTTVSESTKRENMDFSISISNITPADAGTYTCVKF  
 RKGSPDTEFKSGAGTELSVRAPKPSAPVVSGPAARATPQHTVSFTCESHGFSPRDITLKWFKN  
 10 GNELSDFQTNVDPVGESVSYSIHSTAKVVLTRDVDHSQVICEVAHVTLQGDPLRGRTANLSET  
 IRVPPTLEVTQQPVRAENQVNVTCQVRKFYFQRLQLTWLENGNVSRTEASTVTENKDGTYN  
 WMSWLLVNVSAHRDDVKLTQCVEHDGQPAVSKSHDLKVSAPHPKEQGSNTAAENTGSNERNGG  
 GLNDIFEAQKIEWHEIEGRDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV  
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN  
 15 KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP  
 ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:52 (human SIRP $\alpha_{BIT}$ , extracellular domain 1-370, *Avi-FXa-Fc tag*)

MEPAGPAPGRLGPLLCLLLAASCAWSGVAGEEELQVIQPKSVLVAAGETATLRCTATSLIP  
 20 VGPIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLTNRNMDFSIRIGNITPADAGTYTCVKF  
 RKGSPDDVEFKSGAGTELSVRAPKPSAPVVSGPAARATPQHTVSFTCESHGFSPRDITLKWFK  
 NGNELSDFQTNVDPVGESVSYSIHSTAKVVLTRDVDHSQVICEVAHVTLQGDPLRGRTANLSE  
 TIRVPPTLEVTQQPVRAENQVNVTCQVRKFYFQRLQLTWLENGNVSRTEASTVTENKDGTY  
 NWMSWLLVNVSAHRDDVKLTQCVEHDGQPAVSKSHDLKVSAPHPKEQGSNTAAENTGSNERNG  
 25 GGLNDIFEAQKIEWHEIEGRDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV  
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS  
 NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ  
 PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK

30 SEQ ID NO:53 (human SIRP $\beta_{1v1}$ , extracellular domain, *Avi-FXa-Fc tag*)

MPVPASWPHLPSPFLMLTLLGRLTGVADEDELQVIQPEKSVSVAAGESATLRCAMTSLIPV  
 GPIMWFRGAGAGRELIYNQKEGHFPRVTTVSELTKRNNLDFSISISNITPADAGTYTCVKFR  
 KGSPDDVEFKSGAGTELSVRAPKPSAPVVSGPAVRATPEHTVSFTCESHGFSPRDITLKWFKN  
 GNELSDFQTNVDPAGDSVSYSIHSTARVVLTRGDVHSQVICEIAHITLQGDPLRGRTANLSEA

IRVPPTLEVTQQPMRAENQANVTCQVSNFYPRGLQLTWLENGNVSRTETASTLIENKDGTYN  
WMSWLLVNTCAHRDDVVLTCQVEHDGQQAVSKSYALEISAHQKEHGS DITHEAALAPTAPLG  
GGLNDIFEAQKIEWHEIEGRDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV  
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
5 NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ  
PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

**SEQ ID NO:54 (human SIRP $\beta_{1v2}$ , extracellular domain, *Avi-FXa-Fc tag*)**

MPVPASWPHLPSPFLLMTLLLGRLTG VAGEEELQVIQPKDSISVAAGESATLHCTVTSLIPV  
10 GPIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLT KRNNMDFSIRISNITPADAGTY YCVKFR  
KGSPDHVEFKSGAGTELSVRAKPSAPVVS GPAARATPQHTVSFTCESHGFSPR DITLKWFKN  
GNELSD FQTNVDPAGDSVSYSIHSTAKVVL TREDVHSQVICEVAHVTLQGDPLRG TANLSET  
IRVPPTLEVTQQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNVSRTETASTLTENKDGTYN  
WMSWLLVNVSAHRDDVKLT CQVEHDGQPAVSKSHDLKVS AHPKEQGSNTAPGPALASAAPLG  
15 GGLNDIFEAQKIEWHEIEGRDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV  
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ  
PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

**20 SEQ ID NO:55 (human SIRP $\gamma$ , extracellular domain, *Avi-FXa-Fc tag*)**

MPVPASWPHPPGPFLLLTLLGLTEVAGEEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVG  
PVLWFRGVGPGRELIYNQKEGHFPRVTTVSDLT KRNNMDFSIRISSITPADVGTYYCVKFRK  
GSPENVEFKSGPGTEMALGAKPSAPVVLGPAARTTPEHTVSFTCESHGFSPR DITLKWFKNG  
NELSD FQTNVDP TGQSVAYSIRSTARVVLDPWDVRSQVICEVAHVTLQGDPLRG TANLSEAI  
25 RVPPTLEVTQQPMRVGNQVNVTCQVRKFYPQSLQLTWS ENGNVCQRETASTLTENKDGTYNW  
TSWFLVNI SDQRDDVVLTCQVKHDGQLAVSKRLALEVT VHQKDQSSDATPKGQDNSADIQHS  
GGRSSLEGPRFEGKPIPNLLGLDSTRTG GGLNDIFEAQKIEWHEIEGRDKTHTCPPCPAP  
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE  
30 EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQ  
QGNV FSCSV MHEALHNHYTQKSLSLSPGK

SEQ ID NO:56 (cynomolgus monkey SIRP $\alpha$ , extracellular domain, *Avi-FXa-Fc tag*)

MEPAGPAPGRLGPLLCLLLTASCAWSGVLGEEELQVIQPEKSVSVAAGDSATLNCTVSSSLIP  
 VGPIQWFRGAGPGRELIYNLKEGHFPRVTAVSDPTKRNNMDFSIRISNITPADAGTYCYCVKF  
 RKGSPDVELKSGAGTELSVRAKPSAPVVSGPAVRATAEHTVSFTCESHGFSPRDITLKWFKN  
 5 GNELSDVQTNVDPAGKSVSYSIRSTARVLLTRRDVHSQVICEVAHVTLQGDPLRGTTANLSEA  
 IRVPPFLEVTQQSMRADNQVNVTCQVTKFYQRLQLTWLENGNVSRTEMASALPENKDGTYN  
 WTSWLLVNVSAHRDDVKLTQCVEHDGQPAVNKSFVSVKSAHPKEQGSNTAAENTGTNERNGG  
*GLNDIFEAQKIEWHEIEGRDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN*  
*KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP*  
 10 *ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK*

SEQ ID NO:57 (HC; 1H9; including leader sequence [underlined]; constant region *[italic]*)

MACPGFLWALVISTCLEFSMAQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWITWVKQAP  
 15 GQGLEWIGDIYPGSGSTNHIEKFKSKATLTVDTISISTAYMELSRLRSDDTAVYYCATGYGSS  
 YGYFDYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA  
*LTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH*  
*TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN*  
*AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV*  
 20 *YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL*  
*TVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK*

SEQ ID NO:58 (LC; 1H9; including leader sequence [underlined]; constant region *[italic]*)

MACPGFLWALVISTCLEFSMADIQMTQSPSSLSASVGDRVTITCRASENIYSYLAWYQQKPG  
 25 KAPKLLIYTAKTLAEGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQHQQYGPFPFTFGQGTK  
 LEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE  
*QDSKDYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*

SEQ ID NO:59 (HC; 40A-1; including leader sequence [underlined]; constant region *[italic]*)

30 MACPGFLWALVISTCLEFSMAQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWITWVKQAP  
 GQGLEWIGDIYPGSGSTNHIEKFKSKATLTVDTISISTAYMELSRLRSDDTAVYYCATGYGSS  
 YGYFDYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA  
*LTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH*  
*TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN*

AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV  
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKL  
TVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

- 5 SEQ ID NO:60 (LC; 40A-1; including leader sequence [underlined]; constant region *[italic]*)  
MACPGFLWALVISTCLEFSMADIQMTQSPSSLSASVGDRVTITCRASQDIGSRLNWLQQKPG  
KAPKRLIYATSSLDGVPSPRFSGSGSGTEFTLTISSLQPEDFATYYCLQYASSPFTFGGGTK  
VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE  
QDSKDYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

10

- SEQ ID NO:61 (HC; 40A-2; including leader sequence [underlined]; constant region *[italic]*)  
MACPGFLWALVISTCLEFSMAEVQLVQSGAEVVKPGASVKLSCKASGSTFTSYMMHWVKQAP  
GQGLEWIGAIYPVNSDTTYNQKFKGKATLTVDKSASTAYMELSSLRSEDTAVYYCTRSFYYS  
LDAAWFVYWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS  
15 GALTSGVHTFPAVLQSSGLYSLSSVTVPSNFGTQTYTCNVDHKPSNTKVDKTVVERKCCVE  
CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAK  
TKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVY  
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSGDSFFLYSKLTV  
DKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

20

- SEQ ID NO:62 (LC; 40A-2; including leader sequence [underlined]; constant region *[italic]*)  
MACPGFLWALVISTCLEFSMADIQMTQSPSSLSASVGDRVTITCRASQDIGSRLNWLQQKPG  
KAPKRLIYATSSLDGVPSPRFSGSGSGTEFTLTISSLQPEDFATYYCLQYASSPFTFGGGTK  
VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE  
25 QDSKDYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

- SEQ ID NO:63 (HC; AB3-LALA; including leader sequence [underlined]; constant region *[italic]*)

- MACPGFLWALVISTCLEFSMAAVTLDES GGGLQTPGGALSLVCKASGFIFSDYGMNWVRQAP  
30 GKGLEFVAQITSGSRYYGA AVKGRATISRDNRQSTVKLQLNNLRAEDTGIYFCARDFGSGV  
GSIDAWNGNGTEVIVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL  
TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT  
CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA  
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY

TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT  
VDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

SEQ ID NO:64 (LC; AB3-LALA; including leader sequence [underlined]; constant region

5 *[italic]*)

MACPGFLWALVISTCLEFSMAALTQPASVSANLGGTVKITCSGSRGRYGWYQQRSPGSAPVT  
VIYRDNRPSNIPSRFSSSTSGSTSTLTITGVQADDESUYFCGSYDGSIDIFGAGTTTLTVLR  
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD  
STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

10

SEQ ID NO:65 (HC; AB25-LALA; including leader sequence [underlined]; constant region  
*[italic]*)

MACPGFLWALVISTCLEFSMADVQLVESGGGVVRPGESLRLSCEASGFTFSSNAMSWVRQAP  
GKGLEWVAGISSGSDTYYGDSVKGRLTISRDNKNILYLQMNSLTAEDTAVYYCARETWNHL  
15 FDYWGLGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS  
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP  
PCPAPEAAAGGSPVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT  
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL  
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD  
20 KSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

SEQ ID NO:66 (LC; AB25-LALA; including leader sequence [underlined]; constant region  
*[italic]*)

MACPGFLWALVISTCLEFSMASYELTQPPSVSVSPGQTARITCSGGSYSSYYAWYQQKPGQ  
25 APVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYYCGGYDQSSYTNPFGGGT  
KLTVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT  
EQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:67 (HC; AB115-LALA; including leader sequence [underlined]; constant region

30 *[italic]*)

MACPGFLWALVISTCLEFSMAVQLVESGGGVVRPGESLRLSCAASGFSFSSYAMNWVRQAPG  
EGLEWVSRINSGGGGTDYAESVKGRFTISRDNSENTLYLQMNSLRAEDTAVYYCAKQYDWNS  
FFDYWGLGALTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT  
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC

PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY  
LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV  
DKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

5

SEQ ID NO:68 (LC; AB115-LALA; including leader sequence [underlined]; constant region [*italic*])

MACPGFLWALVISTCLEFSMAETVLTQSPATLSVSPGERATLSCRASQTVGSKLAWHQKPG  
QAPRLLIYDATNRATGISDRFSGSGSGTDFTLTITSSQLQTEDSAVYYCQYYWPPYRFGGGT  
10 KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT  
EQDSKDYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:69 (HC; AB119-LALA; including leader sequence [underlined]; constant region [*italic*])

15 MACPGFLWALVISTCLEFSMAVQLLESQGGGVVQPGGSLRLSCAASGFSFSNFMATWVRQAPG  
EGLEWVSTIGSDTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDSTVSWSG  
DFFDYWGLGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL  
TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT  
CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA  
20 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY  
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT  
VDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

SEQ ID NO:70 (LC; AB119-LALA; including leader sequence [underlined]; constant region [*italic*])

25 MACPGFLWALVISTCLEFSMAEIVLTQSPATLSVSPGERATFSCRASQNVKNDLAWYQQRPG  
QAPRLLIYAARIRETGIPERFSGSGSGTEFTLTITSLQSEDFAVYYCQYYDWPPFTFGGGT  
KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT  
EQDSKDYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

30

SEQ ID NO:71 (HC; AB136-LALA; including leader sequence [underlined]; constant region [*italic*])

MACPGFLWALVISTCLEFSMADVQLVESGGGVVVRPGESLRLSCAASGFTFSSYDMNWVRQAP  
GEGLEWVSLISGSGEIIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKENNRY

RFFDDWGLGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL  
 TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT  
 CPPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA  
 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY  
 5 TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT  
 VDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

SEQ ID NO:72 (LC; AB136-LALA; including leader sequence [underlined]; constant region *[italic]*)

10 MACPGFLWALVISTCLEFSMAETVLTQSPGTLTLSPGERATLTCRASQSVYTYLAWYQEKPG  
 QAPRLLIYGASSRATGIPDRFSGSGSGTEFTLTISSLQSEDAFVYYCQQYYDRPPLTFGGGT  
 KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT  
 EQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

15 SEQ ID NO:73 (HC; 3F9-LALA; including leader sequence [underlined]; constant region *[italic]*)

MACPGFLWALVISTCLEFSMAEVLVESGGGLVKGPGSLKLSCAASGFTFSSYAMSWVRQTP  
 EKRLEWVATISDYGGSYTYYPDSVKGRFTISRDNKYTLYLQMSSLRSEDTALYYCARPPYD  
 DYYGGFAYWQGTLTVTSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS  
 20 GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK  
 THTCPPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP  
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS  
 KLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

25

SEQ ID NO:74 (LC; 3F9-LALA; including leader sequence [underlined]; constant region *[italic]*)

MACPGFLWALVISTCLEFSMADIVLTQSPASLAVSLGQRATISCRASKSVSSSGYSYMHYQ  
 QKPGQPPKLLIYLASNLESGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHNRELPTFG  
 30 GGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE  
 SVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:75 (HC; 7H9-LALA; including leader sequence [underlined]; constant region [*italic*])

MACPGFLWALVISTCLEFSMADVQLQESGPGLVKPSQSLSLTCTVTGFSISRGYDWHWIRHF  
 PGNILEWMGYITYSGISNYPNPSLKSRISITHDTSKNHFFLRRLNSVTAEDTATYYCARGGGAW  
 5 FTYWGQGTLLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS  
 GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP  
 PCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT  
 KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL  
 PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD  
 10 KSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

SEQ ID NO:76 (LC; 7H9-LALA; including leader sequence [underlined]; constant region [*italic*])

MACPGFLWALVISTCLEFSMADIVMTQSPATLSVTPGDRVSLSCRASQSIQSLHWYHQKSH  
 15 ESPRLLIKYPASQSIGIPSRFSAGGSGSDFTLTINSVEPEDVGYYCQNGHSLPWTFGGGTK  
 LEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE  
 QDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

20

Table 8. Examples of amino acid residue substitutions in heavy chain FR1

|   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| V | Q | L | V | E | S | G | G | R  | L  | V  | Q  | P  | G  | T  | S  | L  | T  | L  | S  | C  | T  | V  | S  | G  | F  | S  | L  | S  |
| R | S | V | E |   |   |   |   | G  |    | G  |    |    |    | G  | P  |    | R  |    |    |    | A  | A  |    |    |    |    |    |    |
| Q |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

Table 9. Examples of amino acid residue substitutions in heavy chain FR2

|    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 |
| W  | F  | R  | Q  | A  | P  | G  | K  | G  | L  | E  | Y  | I  | G  |
|    | V  |    |    |    |    |    |    |    |    |    | W  | V  |    |

Table 10. Examples of amino acid residue substitutions in heavy chain FR3

|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 |
| R  | F  | T  | I  | S  | K  | T  | S  | T  | T  | M  | D  | L  | K  | M  | N  | S  | L  | R  | S  | E  | D  | T  | A  | T  | Y  | F  | C  | A  | R  |
|    |    |    | G  |    |    |    |    | S  |    |    | A  | Y  | Q  | L  | T  |    | P  | T  | T  |    |    |    | V  |    | Y  |    |    |    |    |

Table 11. Examples of amino acid residue substitutions in heavy chain FR4

|     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 |
| W   | G   | P   | G   | T   | L   | V   | T   | V   | S   | S   |
|     |     | Q   |     |     |     |     |     |     |     |     |

Table 12. Examples of amino acid residue substitutions in light chain FR1

|   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| D | I | Q | M | T | Q | S | P | S | S  | L  | S  | A  | S  | V  | G  | D  | R  | V  | T  | I  | T  | C  |
|   |   | E |   |   |   |   |   |   |    | V  |    |    |    |    |    |    |    |    |    | L  |    |    |
|   |   | V |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

Table 13. Examples of amino acid residue substitutions in light chain FR2

|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 |
| W  | Y  | Q  | Q  | K  | P  | G  | K  | A  | P  | K  | L  | L  | I  | Y  |
|    |    |    |    |    |    |    | Q  |    |    |    |    |    |    |    |

5 Table 14. Examples of amino acid residue substitutions in light chain FR4

|    |    |     |     |     |     |     |     |     |     |
|----|----|-----|-----|-----|-----|-----|-----|-----|-----|
| 98 | 99 | 100 | 101 | 102 | 103 | 104 | 105 | 106 | 107 |
| F  | G  | Q   | G   | T   | K   | V   | E   | I   | K   |
|    |    | G   |     |     |     |     |     |     |     |

**CLAIMS**

1. A humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof, comprising heavy chain complementarity determining regions (HCDR) and light chain complementarity determining regions (LCDR) HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the antibody or the antigen-binding fragment thereof comprises:
  - a. HCDR1 comprising SEQ ID NO:36;
  - b. HCDR2 comprising SEQ ID NO:44;
  - c. HCDR3 comprising SEQ ID NO:45;
  - d. LCDR1 comprising SEQ ID NO:39;
  - e. LCDR2 comprising SEQ ID NO:40; and
  - f. LCDR3 comprising SEQ ID NO:41.
  
2. A humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to claim 1, wherein the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof has one or more properties from the group consisting of:
  - a. the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human SIRP $\alpha_1$  with a binding affinity of at least  $10^{-10}$  M, preferably at least  $10^{-11}$  M, as analysed by surface plasmon resonance at 25°C using human SIRP $\alpha_1$  extracellular domain as shown in SEQ ID NO:51;
  - b. the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human SIRP $\alpha_{BIT}$  with a binding affinity of at least  $10^{-10}$  M, preferably at least  $10^{-11}$  M, as analysed by surface plasmon resonance at 25°C using human SIRP $\alpha_{BIT}$  extracellular domain as shown in SEQ ID NO:52;
  - c. the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds cynomolgus monkey SIRP $\alpha$  with a binding affinity of at least  $10^{-8}$  M, preferably at least  $10^{-9}$  M, as analysed by surface plasmon resonance at 25°C using cynomolgus SIRP $\alpha$  extracellular domain as shown in SEQ ID NO:56;
  - d. the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof does not bind human SIRP $\gamma$  as measured by T-cell binding using flow cytometry, preferably using fluorescence-activated cell sorting staining;
  - e. the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof does not bind human SIRP $\gamma$  as analysed by surface plasmon resonance at 25°C using human SIRP $\gamma$  extracellular domain as shown in SEQ ID NO:55; and

- f. the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof is not immunogenic as determined by IL-2 enzyme-linked immunosorbent spot (ELISpot) and/or T-cell proliferation assay.
- 5     3. A humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to claim 1 or claim 2, wherein:
- 10     a. the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human SIRP $\alpha_1$  with a binding affinity of at least  $10^{-10}$  M, preferably at least  $10^{-11}$  M, as analysed by surface plasmon resonance at 25°C using human SIRP $\alpha_1$  extracellular domain as shown in SEQ ID NO:51;
- 15     b. the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof binds human SIRP $\alpha_{BIT}$  with a binding affinity of at least  $10^{-10}$  M, preferably at least  $10^{-11}$  M, as analysed by surface plasmon resonance at 25°C using human SIRP $\alpha_{BIT}$  extracellular domain as shown in SEQ ID NO:52;
- 20     c. blocks CD47 binding to SIRP $\alpha_1$  and SIRP $\alpha_{BIT}$  as analysed by dissociation from captured CD47 by surface plasmon resonance; and
- 25     d. the anti-SIRP $\alpha$  antibody or the antigen-binding fragment thereof does not bind human SIRP $\gamma$  as measured by T-cell flow cytometry staining, preferably fluorescence-activated cell sorting staining.
- 30     4. A humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to any one of the preceding claims, wherein:
- 35     a. the heavy chain variable domain of the antibody comprises 4 heavy chain framework regions, HFR1 to HFR4, and 3 complementarity determining regions HCDR1 to HCDR3 that are operably linked in the order HFR1-HCDR1-HFR2-HCDR2-HFR3-HCDR3-HFR4, wherein each of the heavy chain framework regions has at least 90% amino acid identity with the framework amino acid sequence of SEQ ID NO:8, or wherein HFR1 to HFR4 differ from SEQ ID NO:8 in one or more of the amino acid substitutions as defined in Tables 8 to 11; and
- 40     b. the light chain variable domain of the antibody comprises 4 light chain framework regions, LFR1 to LFR4, and 3 complementarity determining regions LCDR1 to LCDR3 that are operably linked in the order LFR1-LCDR1-LFR2-LCDR2-LFR3-LCDR3-LFR4, wherein each of the light chain framework regions has at least 90% amino acid identity with the framework amino acid

sequence of SEQ ID NO:9, or wherein LFR1, LFR2 and/or LFR4 differ from SEQ ID NO:9 in one or more amino acid substitutions as defined in Tables 12 to 14.

- 5     5.     A humanized anti-SIRP $\alpha$  antibody or an antigen-binding fragment thereof according to any one of the preceding claims, comprising a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the antibody or the antigen-binding fragment thereof comprises the HCVR amino acid sequence of SEQ ID NO:8 and the LCVR amino acid sequence of SEQ ID NO:9.
- 10     6.     A humanized anti-SIRP $\alpha$  antibody according to any one of the preceding claims, 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.
- 15     7.     A humanized anti-SIRP $\alpha$  antibody according to any one of the preceding claims, comprising a modified human IgG<sub>1</sub> Fc region, comprising an amino acid substitution at one or more positions selected from the group consisting of L234, L235, G237, D265, D270, N297, A327, P328, and P329 according to Eu numbering.
- 20     8.     A humanized anti-SIRP $\alpha$  antibody according to claim 7 comprising the amino acid substitutions L234A and L235A; L234E and L235A; L234A, L235A and P329A; or L234A, L235A and P329G.
- 25     9.     A pharmaceutical composition comprising a humanized anti-SIRP $\alpha$  antibody according to any one of claims 1 – 8, or an antigen-binding fragment thereof according to any one of claims 1 – 5, and a pharmaceutically acceptable excipient.
- 30     10.    A humanized anti-SIRP $\alpha$  antibody according to any one of claims 1 – 8, an antigen-binding fragment thereof according to any one of claims 1 – 5, or a pharmaceutical composition according to claim 9 for use as a medicament.
11.    A humanized anti-SIRP $\alpha$  antibody according to any one of claims 1 – 8, an antigen-binding fragment thereof according to any one of claims 1 – 5 or a pharmaceutical

composition according to claim 9 for use in the treatment of a cancer, wherein the treatment further comprises administration of a therapeutic antibody, wherein the cancer preferably is a human solid tumour or a haematological malignancy.

- 5      12. A humanized anti-SIRP $\alpha$  antibody according to any one of claims 1 – 8, an antigen-binding fragment thereof according to any one of claims 1 – 5 or a pharmaceutical composition according to claim 9 for a use according to claim 11, wherein the therapeutic antibody is directed against a membrane-bound target on the surface of tumour cells and comprises a human Fc region that binds to activating Fc receptors present on human  
10      immune effector cells.
13. A humanized anti-SIRP $\alpha$  antibody according to any one of claims 1 – 8, an antigen-binding fragment thereof according to any one of claims 1 – 5 or a pharmaceutical composition according to claim 9 for a use according to claim 11 or 12, wherein the  
15      human solid tumour is selected from the group consisting of (HER2-positive) breast cancer, (EGFR-positive) colon carcinoma, (GD2-positive) neuroblastoma, melanoma, osteosarcoma, (CD20-positive) B-cell lymphomas, (CD38-positive) multiple myeloma (CD52-positive) lymphoma, (CD33-positive) acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML), chronic lymphatic leukaemia (CLL), acute lymphoblastic  
20      leukaemia (ALL), non-Hodgkin's lymphoma (NHL), including follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL), hepatocellular carcinoma, multiple myeloma (MM), bladder cancer, gastric cancer, ovarian cancer, head and neck cancer, pancreatic cancer, renal carcinoma, prostate cancer, hepatocellular carcinoma and lung cancer.
- 25      14. A humanized anti-SIRP $\alpha$  antibody according to any one of claims 1 – 8, an antigen-binding fragment thereof according to any one of claims 1 – 5 or a pharmaceutical composition according to claim 9 for a use according to any one of claims 11 – 13, wherein the treatment comprises administration of a further anti-cancer therapeutic  
30      compound.
15. A humanized anti-SIRP $\alpha$  antibody according to any one of claims 1 – 8, an antigen-binding fragment thereof according to any one of claims 1 – 5 or a pharmaceutical composition according to claim 9 for a use according to claim 14, wherein the further

anti-cancer therapeutic compound is a targeted therapeutic, preferably an immunotherapeutic agent.

16. A nucleic acid molecule comprising a nucleotide sequence encoding a humanized anti-SIRP $\alpha$  antibody according to any one of claims 1 – 8, or an antigen binding fragment thereof according to any one of claims 1 – 5, wherein preferably the nucleic acid molecule comprises a nucleotide sequence encoding at least one of the HCVR and the LCVR of the antibody, and wherein preferably the coding nucleotide sequence is operably linked to regulatory sequences for expression of the coding nucleotide sequence in a host cell.
17. A host cell comprising the nucleic acid molecule according to claim 16.