

for undesirable side effects, and which anti-SIRP α antibodies bind to both human SIRP α_1 and human SIRP α_{BIT} polymorphic variants, to make them suitable for a large part of the human population, including SIRP α_1 /SIRP α_{BIT} heterozygotes, SIRP α_{BIT} homozygotes and SIRP α_1 homozygotes. There is a need for anti-SIRP α antibodies with these characteristics and which
5 reduce inhibitory, i.e. SHP-1 and/or SHP-2-mediated, SIRP α signalling. Such antibodies are suitable for use in anti-cancer therapy either alone or, preferably, in combination with a therapeutic anti-cancer antibody.

SUMMARY

10 The present disclosure describes humanized antibodies against SIRP α 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 disclosure refers to a humanized anti-SIRP α 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 one example, the anti-SIRP α antibody or the antigen-binding fragment thereof has one
25 or more properties from the group consisting of: (a) the anti-SIRP α antibody or the antigen-binding fragment thereof binds human SIRP α_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 BiaCoreTM) at 25°C using human SIRP α_1 extracellular domain as shown in SEQ ID NO:51; (b) the anti-SIRP α antibody or the antigen-binding fragment thereof binds human SIRP α_{BIT} with a
30 binding affinity of at least 10^{-10} M, preferably at least 10^{-11} M, as analysed by SPR (preferably BiaCoreTM) at 25°C using human SIRP α_{BIT} extracellular domain as shown in SEQ ID NO:52; (c) the anti-SIRP α antibody or the antigen-binding fragment thereof binds cynomolgus monkey SIRP α with a binding affinity of at least 10^{-8} M, preferably at least 10^{-9} M, as analysed by SPR (preferably BiaCoreTM) at 25°C using cynomolgus SIRP α extracellular

domain as shown in SEQ ID NO:56; (d) the anti-SIRP α antibody or the antigen-binding fragment thereof does not bind human SIRP γ as measured by T-cell binding using flow cytometry, preferably using fluorescence-activated cell sorting (FACS) staining; (e) the anti-SIRP α antibody or the antigen-binding fragment thereof does not bind human SIRP γ as analysed by SPR (preferably BiaCoreTM) at 25°C using human SIRP γ extracellular domain as shown in SEQ ID NO:55; and (f) the anti-SIRP α 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 another example, the humanized anti-SIRP α antibody or an antigen-binding fragment thereof: (a) binds human SIRP α_1 with a binding affinity of at least 10^{-10} M, preferably at least 10^{-11} M, as analysed by SPR (preferably BiaCoreTM) at 25°C using human SIRP α_1 extracellular domain as shown in SEQ ID NO:51; (b) binds human SIRP α_{BIT} with a binding affinity of at least 10^{-10} M, preferably at least 10^{-11} M, as analysed by SPR (preferably BiaCoreTM) at 25°C using human SIRP α_{BIT} extracellular domain as shown in SEQ ID NO:52; (c) blocks CD47 binding to SIRP α_1 and SIRP α_{BIT} , preferably as analysed by dissociation from captured CD47 by SPR (preferably BiaCoreTM), more preferably as described in Example 6; and (d) does not bind human SIRP γ as measured by T-cell flow cytometry, preferably fluorescence-activated cell sorting (FACS) staining.

In yet another example, there is disclosed a humanized anti-SIRP α 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 another example, a humanized anti-SIRP α antibody or an antigen-binding fragment thereof according to the disclosure, comprises a heavy chain variable region (HCVR)

amino acid sequence and a light chain variable region (LCVR), wherein the anti-SIRP α 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.

5 In another example, a humanized anti-SIRP α antibody according to the disclosure, comprises a modified Fc region that exhibits reduced binding to a human Fc α or Fc γ receptor compared to the same anti-SIRP α 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 example, a modified human IgG₁ Fc region comprises 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
10 numbering. More preferably, a modified human IgG₁ 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 yet another example, the human IgG Fc region does not comprise other amino acid substitutions.

15 In a second aspect, the present disclosure refers to a pharmaceutical composition comprising a humanized anti-SIRP α antibody, or an antigen-binding fragment thereof, as disclosed herein and a pharmaceutically acceptable excipient.

In a third aspect, the present disclosure refers to a humanized anti-SIRP α antibody, or an antigen-binding fragment thereof, as disclosed herein, or a pharmaceutical composition as disclosed
20 herein for use as a medicament.

In a fourth aspect, the present disclosure refers to a use of a humanized anti-SIRP α antibody or an antigen-binding fragment thereof as disclosed herein or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treatment of a cancer, wherein the treatment further comprises a therapeutic antibody to be administered, wherein the cancer preferably is a human solid
25 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 one example, the 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)
30 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 another example, the treatment comprises of a further anti-cancer therapeutic compound to be administered, such as for example a targeted therapeutic, preferably an immunotherapeutic agent.

In a fifth aspect, the present disclosure refers to a nucleic acid molecule comprising a nucleotide sequence encoding a humanized anti-SIRP α antibody or an antigen binding fragment thereof as disclosed herein, 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.

In a sixth aspect, the present disclosure refers to a host cell comprising the nucleic acid molecule as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Binding of antibodies to SIRP γ expressing human CD3⁺ 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 individuals.

Figure 2. Comparison of the ADCC measured in % cytotoxicity of trastuzumab (Tmab) alone, trastuzumab in combination with the murine 12C4 anti-SIRP α antibody (mu12C4), trastuzumab in combination with an antibody wherein murine 12C4 variable regions are grafted onto the human IgG₁ constant region (12C4huIgG₁), and trastuzumab in combination with an antibody wherein murine 12C4 variable regions are grafted onto the human IgG₁ constant region comprising the amino acid substitutions L234A and L235A (12C4huIgG₁LALA; 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 α _{BIT} alleles. Increasing concentrations of trastuzumab were employed: 0, 0.05, 0.5 and 5 μ g/ml, respectively, as well as increasing concentrations of each anti-SIRP α antibody (0.2, 2 and 20 μ g/ml, respectively).

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

CLAIMS

1. A humanized anti-SIRP α 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,
5 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;
 - 10 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 α antibody or an antigen-binding fragment thereof according to
15 claim 1, wherein the anti-SIRP α antibody or the antigen-binding fragment thereof has one or more properties from the group consisting of:
 - a. the anti-SIRP α antibody or the antigen-binding fragment thereof binds human SIRP α_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 α_1
20 extracellular domain as shown in SEQ ID NO:51;
 - b. the anti-SIRP α antibody or the antigen-binding fragment thereof binds human SIRP α_{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 α_{BIT} extracellular domain as shown in SEQ ID NO:52;
 - 25 c. the anti-SIRP α antibody or the antigen-binding fragment thereof binds cynomolgus monkey SIRP α 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 α extracellular domain as shown in SEQ ID NO:56;
 - d. the anti-SIRP α antibody or the antigen-binding fragment thereof does not bind
30 human SIRP γ as measured by T-cell binding using flow cytometry, preferably using fluorescence-activated cell sorting staining;
 - e. the anti-SIRP α antibody or the antigen-binding fragment thereof does not bind human SIRP γ as analysed by surface plasmon resonance at 25°C using human SIRP γ extracellular domain as shown in SEQ ID NO:55; and

- f. the anti-SIRP α 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.
3. A humanized anti-SIRP α antibody or an antigen-binding fragment thereof according to claim 1 or claim 2, wherein:
 - a. the anti-SIRP α antibody or the antigen-binding fragment thereof binds human SIRP α_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 α_1 extracellular domain as shown in SEQ ID NO:51;
 - b. the anti-SIRP α antibody or the antigen-binding fragment thereof binds human SIRP α_{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 α_{BIT} extracellular domain as shown in SEQ ID NO:52;
 - c. blocks CD47 binding to SIRP α_1 and SIRP α_{BIT} as analysed by dissociation from captured CD47 by surface plasmon resonance; and
 - d. the anti-SIRP α antibody or the antigen-binding fragment thereof does not bind human SIRP γ as measured by T-cell flow cytometry staining, preferably fluorescence-activated cell sorting staining.
 4. A humanized anti-SIRP α antibody or an antigen-binding fragment thereof according to any one of the preceding claims, 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.

- 5 5. A humanized anti-SIRP α 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 α antibody according to any one of the preceding claims, comprising a modified Fc region that exhibits reduced binding to a human Fc α or Fc γ receptor compared to the same anti-SIRP α antibody comprising a wild-type Fc region.
- 15 7. A humanized anti-SIRP α antibody according to any one of the preceding claims, comprising a modified human IgG₁ 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 α 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 α 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 α 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. Use of a humanized anti-SIRP α 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 in the manufacture of a medicament for the treatment of a cancer, wherein the treatment further comprises a therapeutic antibody to be administered, wherein the cancer preferably is a human solid tumour or a haematological malignancy.

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12. The 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 immune effector cells.

10 13. The use according to claim 11 or 12, wherein the 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-
15 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.

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14. The use according to any one of claims 11 – 13, wherein the treatment comprises a further anti-cancer therapeutic compound to be administered.

15. The use according to claim 14, wherein the further anti-cancer therapeutic compound is
25 a targeted therapeutic, preferably an immunotherapeutic agent.

16. A nucleic acid molecule comprising a nucleotide sequence encoding a humanized anti-SIRP α 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
30 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.