

studies have demonstrated that antibody-dependent cell-mediated cytotoxicity (ADCC) is the predominant mode of action against cancer cells. The requirement for a direct interaction between Ab and receptors for the Fc region of the antibodies (FcR) has been demonstrated for anti-tumor effects of these antibodies. Consequently, FcR-bearing immune effector cells play an important role in mediating their effects (Iannello A. and Ahmad A., 2005). However, MIF antibodies known in the art neither induce complement-dependent cell lysis of MIF-responsive cells nor antibody-dependent cellular cytotoxicity (Hussain et al., 2013).

US2011/236375A1 describes Fc variants with enhanced effector function.

A combination of reduced aggregation potential and reduced hydrophobicity would render the antibodies' properties highly advantageous. There is a need in the art for aggregation-resistant antibodies against oxMIF with reduced hydrophobicity but also for anti-oxMIF antibodies with enhanced effector functions, which has not yet been met by current antibody modifications.

SUMMARY OF THE INVENTION

It is the objective of the present inventiondisclosure to provide improved antibodies or antigen binding fragments thereof targeting oxMIF and having reduced aggregation propensity and hydrophobicity.

The objective is solved by the subject matter of the present inventiondisclosure.

The present inventiondisclosure discloses a recombinant anti-oxMIF antibody or an antigen binding fragment thereof having reduced aggregation potential and reduced hydrophobicity, comprising the following variable domains:

(a1) a light chain variable domain comprising SEQ ID NO:9 and at least one of the amino acid substitutions M30L, F49Y, A51G, P80S, W93F, or

(a2) a light chain variable domain comprising SEQ ID NO:9 with 1, 2, 3, 4, or 5 amino acid substitutions, further comprising a conserved tyrosine at position 36, and at least one of the amino acid substitutions M30L, F49Y, A51G, P80S, W93F; and one of

(b1) a heavy chain variable domain comprising SEQ ID NO:6; or

(b2) a heavy chain variable domain comprising SEQ ID NO:6 and the amino acid substitutions L5Q and/or W97Y, or

(b3) a heavy chain variable domain comprising SEQ ID NO:6 with 1, 2, 3, 4 or 5 amino acid substitutions and the amino acid substitutions L5Q and/or W97Y; wherein amino acid positions are numbered according to Kabat, and wherein the aggregation

potential and hydrophobicity are reduced compared to an antibody or antigen binding fragment thereof comprising SEQ ID NO:6 and SEQ ID NO:9 lacking the amino acid substitutions.

In one aspect, the present disclosure refers to a recombinant anti-oxMIF antibody or an antigen binding fragment thereof having reduced aggregation potential and reduced hydrophobicity, comprising the following variable domains:

(a1) a light chain variable domain comprising SEQ ID NO:9 with 1, 2, 3, 4, or 5 amino acid substitutions selected from M30L, F49Y, A51G, P80S and W93F, or

(a2) a light chain variable domain comprising SEQ ID NO:9 with 1, 2, 3, 4, or 5 amino acid substitutions selected from M30L, F49Y, A51G, P80S and W93F, and with 1, 2, 3, 4, or 5 further amino acid substitutions, with the proviso that the tyrosine at position 36 is preserved, and one of

(b1) a heavy chain variable domain comprising SEQ ID NO:6; or

(b2) a heavy chain variable domain comprising SEQ ID NO:6 with 1 or 2 amino acid substitutions selected from L5Q and W97Y, or

(b3) a heavy chain variable domain comprising SEQ ID NO:6 with 1 or 2 amino acid substitutions selected from L5Q and W97Y, and with 1, 2, 3, 4 or 5 further amino acid substitutions,

wherein amino acid positions are numbered according to Kabat, and wherein aggregation potential and hydrophobicity are reduced compared to an antibody or an antigen binding fragment thereof comprising SEQ ID NO:6 and SEQ ID NO:9 lacking the amino acid substitutions.

Herein disclosed is also a recombinant anti-oxMIF antibody or an antigen binding fragment thereof having reduced aggregation potential and reduced hydrophobicity, comprising the following variable domains

(a) a light chain variable domain comprising SEQ ID NO:9 or a light chain variable domain with at least 95% sequence identity to SEQ ID NO:9 comprising a tyrosine at position 36 and

(b) a heavy chain variable domain comprising SEQ ID NO:6 and amino acid substitutions W97Y and/or L5Q, or a heavy chain variable domain comprising SEQ ID NO:6 with 1, 2, 3, 4 or 5 amino acid substitutions and further comprising amino acid substitutions W97Y and/or L5Q, wherein amino acid positions are numbered according to Kabat, and wherein the aggregation potential and hydrophobicity are reduced compared to an antibody or antigen binding fragment thereof comprising SEQ ID NO:6 and SEQ ID NO:9 lacking the amino acid substitutions.

Specifically, the recombinant anti-oxMIF antibody comprises the amino acid substitution W93F with reference to SEQ ID 9.

Specifically, the recombinant anti-oxMIF antibody comprises the amino acid substitution W97Y with reference to SEQ ID NO:6.

According to a further specific embodimentexample, the recombinant anti-oxMIF antibody comprises the amino acid substitutions W93F and W97Y.

Surprisingly, it had been shown that selected positions even within the CDR domains could be substituted to reduce aggregation potential and/or hydrophobicity without having any negative effect on the oxMIF binding affinity.

Specifically, the light chain variable domain comprises 1, 2, 3, 4 or all of the amino acid substitutions M30L, F49Y, A51G, P80S, W93F.

Specifically, the recombinant anti-oxMIF antibody comprises a constant light (CL) domain of SEQ ID NO: 38.

According to a specific embodimentexample, the recombinant anti-oxMIF antibody described herein also has enhanced effector functions and comprises a heavy chain constant region comprising

(a) SEQ ID NO:2 or a heavy chain constant region having at least 95% sequence identity to SEQ ID NO:2 and comprising amino acids 239D and 332E; or

(b) SEQ ID NO:3, or a heavy chain constant region having at least 95% sequence identity to SEQ ID NO:3 and comprising amino acids 239D, 268F, 324T and 332E; or

(c) SEQ ID NO:4, or a heavy chain constant region having at least 95% sequence identity to SEQ ID NO:4 and comprising amino acids 239D, and 332E; or

5 (d) SEQ ID NO:5, or a heavy chain constant region having at least 95% sequence identity to SEQ ID NO:5 and comprising amino acids 235V, 243L, 292P, 300L, and 396L, wherein amino acid positions are numbered according to EU numbering index

Specifically, the anti-oxMIF antibody described herein contains a VH or VL domain containing an amino acid sequence selected from the group consisting of SEQ
10 ID NOs:7, 8, 10, 11, 12, and 13.

According to an alternative embodimentexample, the anti-oxMIF antibody contains SEQ ID NOs:6, 11 and any one of SEQ ID NOs:1, 2, 3, 4 or 5.

In a further embodimentexample, the anti-oxMIF antibody contains SEQ ID NOs:7, 10 and any one of SEQ ID NOs:1, 2, 3, 4 or 5.

15 In a further embodimentexample, the anti-oxMIF antibody contains SEQ ID NOs:7, 11 and any one of SEQ ID NOs:1, 2, 3, 4 or 5.

In a further embodimentexample, the anti-oxMIF antibody contains SEQ ID NOs:8, 10 and any one of SEQ ID NOs:1, 2, 3, 4, or 5.

In a further embodimentexample, the anti-oxMIF antibody contains SEQ ID
20 NOs:8, 11 and any one of SEQ ID NOs:1, 2, 3, 4, or 5, or SEQ ID No:39, or SEQ ID NO:40.

In a further embodimentexample, the anti-oxMIF antibody contains SEQ ID NOs:8, 13 and any one of SEQ ID NOs:1, 2, 3, 4, or 5, or SEQ ID No:41, or SEQ ID
NO:42.

25 Further provided herein is the anti-oxMIF antibody of any one of claims 1 to 3, comprising SEQ ID NOs:39, 40, 41 or 42 or the anti-oxMIF antibody comprising SEQ ID NOs: 39, 44, 41 or 45

More specifically, the recombinant anti-oxMIF antibody or an antigen binding fragment thereof is a bispecific antibody.

30 In a specific embodimentexample, the anti-oxMIF antibody described herein is selected from the group consisting of monospecific and bispecific antibodies, scFv, (scFv)₂, scFv-Fc, Fab, Fab', and F(ab')₂, Fab'-SH, Fab-scFv fusion, Fab-(scFv)₂-fusion, Fab-scFv-Fc, Fab-(scFv)₂-Fc, fusion proteins of two single-chain variable fragments (scFvs) of different antibodies (e.g. BiTE), minibody, TandAb®, DutaMab™, DART, and
35 CrossMab®.

Specifically, the antibody as described herein is for use in the preparation of a medicament. In one aspect, the present disclosure refers to any one of the antibodies described herein for use in the preparation of a medicament.

Specifically, a pharmaceutical composition is provided herein, comprising the antibody of the ~~invention~~disclosure, optionally together with a pharmaceutical carrier or adjuvant. In one aspect, the present disclosure refers to a pharmaceutical composition comprising any one of the antibodies described herein, optionally together with a pharmaceutical carrier or adjuvant.

Specifically, the composition comprises about 10 to 250 mg/ml of the antibody described herein, specifically comprising ≥ 50 mg/ml.

Specifically, said pharmaceutical composition is formulated for subcutaneous administration.

More specifically, the pharmaceutical composition can be used for the treatment of oxMIF-related conditions.

Specifically, the composition is for administration as the sole substance, or in combination with a further pharmaceutical composition comprising one or more active substances, preferably selected from the group consisting of antiviral, anti-inflammatory, anti-cancer, anti-angiogenic and antibiotic substances.

In a further ~~embodiment~~example, the pharmaceutical composition is for use in the treatment of a patient suffering from inflammatory disease, hyperproliferative disorder, infectious disease or cancer, specifically in the treatment of asthma, vasculitis, arthritis, sepsis, septic shock, endotoxic shock, toxic shock syndrome, acquired respiratory distress syndrome, glomerulonephritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, peritonitis, nephritis and psoriasis, colorectal cancer, ovarian cancer, breast cancer, prostate cancer, pancreas cancer, and lung cancer.

In one aspect, the present disclosure refers to use of any one of the pharmaceutical compositions described herein in the manufacture of a medicament for the treatment of a patient suffering from inflammatory disease, hyperproliferative disorder, infectious disease or cancer, specifically in the treatment of asthma, vasculitis, arthritis, sepsis, septic shock, endotoxic shock, toxic shock syndrome, acquired respiratory distress syndrome, glomerulonephritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, peritonitis, nephritis and psoriasis, colorectal cancer, ovarian cancer, breast cancer, prostate cancer, pancreas cancer, and lung cancer.

Further provided herein is an isolated nucleic acid encoding the antibody of the ~~invention~~disclosure. In one aspect, the present disclosure refers to an isolated nucleic acid encoding any one of the antibodies described herein.

~~Also—In one aspect, the present disclosure refers to~~ an expression vector ~~is provided herein~~, comprising the nucleic acid molecule(s) described herein.

In a further ~~embodiment~~aspect, ~~the present disclosure refers to~~ a host cell ~~is provided~~, containing the nucleic acid or an expression vector described herein.

Further provided is a method of producing the antibody of the ~~invention~~disclosure, comprising culturing a host cell and recovering said antibody from the cell culture. In one aspect, the present disclosure refers to a method of producing any one of the antibodies described herein, comprising culturing a host cell described herein and recovering said antibody from the cell culture.

Further provided herein is a method for producing an antibody of the ~~invention~~disclosure, comprising expressing a nucleic acid encoding the antibody in a host cell.

FIGURES

Figure 1: Amino acid sequences

CLAIMS

1. A recombinant anti-oxMIF antibody or an antigen binding fragment thereof having reduced aggregation potential and reduced hydrophobicity, comprising the following variable domains:
- 5 (a1) a light chain variable domain comprising SEQ ID NO:9 with 1, 2, 3, 4, or 5 amino acid substitutions selected from M30L, F49Y, A51G, P80S and W93F, or
- (a2) a light chain variable domain comprising SEQ ID NO:9 with 1, 2, 3, 4, or 5 amino acid substitutions selected from M30L, F49Y, A51G, P80S and W93F, and with 1, 2, 3, 4, or 5 further amino acid substitutions, with the proviso that the tyrosine at
- 10 position 36 is preserved, and one of
- (b1) a heavy chain variable domain comprising SEQ ID NO:6; or
- (b2) a heavy chain variable domain comprising SEQ ID NO:6 with 1 or 2 amino acid substitutions selected from L5Q and W97Y, or
- (b3) a heavy chain variable domain comprising SEQ ID NO:6 with 1 or 2 amino
- 15 acid substitutions selected from L5Q and W97Y, and with 1, 2, 3, 4 or 5 further amino acid substitutions,
- wherein amino acid positions are numbered according to Kabat, and wherein aggregation potential and hydrophobicity are reduced compared to an antibody or an antigen binding fragment thereof comprising SEQ ID NO:6 and SEQ ID NO:9 lacking
- 20 the amino acid substitutions.
2. The recombinant anti-oxMIF antibody of claim 1, comprising the amino acid substitutions W93F and/or W97Y.
- 25 3. The recombinant anti-oxMIF antibody of claim 1 or 2, having enhanced effector functions, comprising a heavy chain constant region comprising
- (a) SEQ ID NO:2 or a heavy chain constant region having at least 95% sequence identity to SEQ ID NO:2 and comprising amino acids 239D and 332E; or
- (b) SEQ ID NO:3, or a heavy chain constant region having at least 95%
- 30 sequence identity to SEQ ID NO:3 and comprising amino acids 239D, 268F, 324T and 332E; or
- (c) SEQ ID NO:4, or a heavy chain constant region having at least 95% sequence identity to SEQ ID NO:4 and comprising amino acids 239D, and 332E; or

(d) SEQ ID NO:5, or a heavy chain constant region having at least 95% sequence identity to SEQ ID NO:5 and comprising amino acids 235V, 243L, 292P, 300L, and 396L,

5 wherein amino acid positions are numbered according to EU numbering index.

4. The anti-oxMIF antibody of any one of claims 1 to 3, comprising SEQ ID NO:8 and an amino acid sequence selected from the group consisting of SEQ ID NOs:10, 11, 12, and 13.

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5. The anti-oxMIF antibody of any one of claims 1 to 2, comprising SEQ ID NOs:6, 11 and any one of SEQ ID NOs:1, 2, 3, 4, or 5.

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6. The anti-oxMIF antibody of any one of claims 1 to 2, comprising SEQ ID NOs:7, 10 and any one of SEQ ID NOs:1, 2, 3, 4, or 5.

7. The anti-oxMIF antibody of any one of claims 1 to 2, comprising SEQ ID NOs:7, 11 and any one of SEQ ID NOs:1, 2, 3, 4, or 5.

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8. The anti-oxMIF antibody of any one of claims 1 to 2, comprising SEQ ID NOs:8, 11 and any one of SEQ ID NOs:1, 2, 3, 4, or 5.

9. The anti-oxMIF antibody of any one of claims 1 to 2, comprising SEQ ID NOs:8, 13 and any one of SEQ ID NOs:1, 2, 3, 4, or 5.

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10. The anti-oxMIF antibody of any one of claims 1 to 2, comprising SEQ ID NOs:8, 10 and any one of SEQ ID NOs:1, 2, 3, 4, or 5.

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11. The anti-oxMIF antibody of any one of claims 1 to 3, comprising SEQ ID NOs:8, 11, 4, and 38.

12. The anti-oxMIF antibody of any one of claims 1 to 3, comprising SEQ ID NOs: 8, 13, 4, and 38.

13. The anti-oxMIF antibody according to any one of claims 1 to 12, selected from the group consisting of bispecific antibodies, scFv, (scFv)₂, scFvFc, Fab, Fab', and F(ab')₂, Fab'-SH, Fab-scFv fusion, Fab-(scFv)₂-fusion, Fab-scFv-Fc, Fab-(scFv)₂-Fc, fusion proteins of two single chain antibodies of different species (BiTE), and minibody.

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14. The antibody of any one of claims 1 to 13 for use in the preparation of a medicament.

15. A pharmaceutical composition comprising the antibody of any one of claims 1 to 13, optionally together with a pharmaceutical carrier or adjuvant.

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16. The pharmaceutical composition according to claim 15, wherein the composition comprises 10 to 250 mg/ml of the antibody of any one of claims 1 to 13, specifically comprising > 50 mg/ml.

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17. The pharmaceutical composition according to claim 15 or 16, wherein said pharmaceutical composition is formulated for subcutaneous administration.

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18. The pharmaceutical composition according to any one of claims 15 to 17, wherein the composition is for administration as the sole substance, or with a further pharmaceutical composition comprising one or more active substances, preferably selected from the group consisting of antiviral, anti-inflammatory, anti-cancer, anti-angiogenic, and antibiotic substances.

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19. Use of ~~the~~ the pharmaceutical composition according to any one of claims 15 to 18 in the manufacture of a medicament for ~~use~~ the treatment of a patient suffering from inflammatory disease, hyperproliferative disorder, infectious disease or cancer, specifically in the treatment of asthma, vasculitis, arthritis, sepsis, septic shock, endotoxic shock, toxic shock syndrome, acquired respiratory distress syndrome, glomerulonephritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, peritonitis, nephritis and psoriasis, colorectal cancer, ovarian cancer, breast cancer, prostate cancer, pancreas cancer, and lung cancer.

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20. An isolated nucleic acid encoding the antibody of any one of claims 1 to 13.

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21. An expression vector comprising the nucleic acid molecule(s) of claim 20.

5 22. A host cell containing the nucleic acid of claim 20 or an expression vector of claim 21.

23. A method of producing the antibody of any one of claims 1 to 13, comprising culturing a host cell of claim 22 and recovering said antibody from the cell culture.

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