Strohl W.R. et al. (2012) describe antibody Fc engineering to increase or decrease FcγR mediated activities of IgG isotypes.

WO2017/178493A1 describes antibodies targeting TIM-3 (T-cell immunoblobulin and mucin domain containing 3) comprising modified CH1-CH3 domains for Fc silencing.A combination of reduced aggregation potential, reduced hydrophobicity and Fc silencing would render the antibody properties highly advantageous. There is still an unmet need in the art for Fc silenced and aggregation-resistant antibodies against oxMIF with reduced hydrophobicity.

SUMMARY

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

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

The present disclosure discloses an Fc silenced anti-oxMIF antibody or an antigen binding fragment thereof, comprising a variant Fc region of a wild-type human IgG comprising SEQ ID NO:1 (CH2-CH3) or, as an alternative, SEQ ID NO:44 (CH1-CH3) with one or more amino acid substitutions or glycosylation modifications, and the following variable domains:

(a1) a light chain variable domain comprising SEQ ID NO:2 having at least one of amino acid substitutions M30L, F49Y, A51G, P80S, W93F, or

(a2) a light chain variable domain comprising SEQ ID NO:2 with 1, 2, 3, 4, or 5 amino acid substitutions further comprising

- a conserved tyrosine at position 36, and
- at least one of amino acid substitutions M30L, F49Y, A51G, P80S, W93F; and

(b1) a heavy chain variable domain comprising SEQ ID NO:3; or

(b2) a heavy chain variable domain comprising SEQ ID NO:3 and the amino acid substitutions L5Q and/or W97Y, or

(b3) a heavy chain variable domain comprising SEQ ID NO:3 having at least one of amino acid substitutions L5Q or W97Y, and 1, 2, 3, 4 or 5 further amino acid substitutions;

wherein amino acid positions are numbered according to Kabat,

wherein the variant Fc region exhibits decreased FcγR binding compared to the wildtype IgG1 Fc region, and

wherein said antibody or antigen binding fragment thereof has reduced aggregation potential and reduced hydrophobicity compared to an antibody or antigen binding fragment thereof comprising SEQ ID NO:2 and SEQ ID NO:3 lacking the amino acid substitutions.

In one aspect, the present disclosure refers to an Fc silenced anti-oxMIF antibody, comprising a variant Fc region of a wild-type human IgG comprising SEQ ID NO:1 with one or more amino acid substitutions or glycosylation modifications, and the following variable domains:

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

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

- conserved tyrosine at position 36 is preserved,

and

(b1) a heavy chain variable domain comprising SEQ ID NO:3; or

(b2) a heavy chain variable domain comprising SEQ ID NO:3 with 1 or 2 amino acid substitutions selected from L5Q and W97Y, or

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

wherein amino acid positions are numbered according to Kabat,

wherein the variant Fc region exhibits decreased FcγR binding compared to the wildtype IgG1 Fc region, wherein the one or more amino acid substitutions in the variant Fc region are at any one of positions E233, L234, L235, G236, G237, P238, D265, S267, H268, N297, S298, T299, E318, L328, P329, A330, P331 of SEQ ID NO:1, specifically the amino acid substitutions are at positions L234 and L235, according to EU numbering index, and/or wherein the Fc region is aglycosylated , and

wherein said antibody has reduced aggregation potential and reduced hydrophobicity compared to an antibody comprising SEQ ID NO:2 and SEQ ID NO:3 lacking the amino acid substitutions.

Specifically, the recombinant anti-oxMIF antibody comprises the amino acid substitution W93F with reference to SEQ ID 2.

Specifically, the recombinant anti-oxMIF antibody comprises the amino acid substitution W97Y with reference to SEQ ID NO:3.

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

According to a specific example described herein, the amino acid substitutions in the Fc regions are at any one of positions E233, L234, L235, G236, G237, P238, D265, S267, H268, N297, S298, T299, E318, L328, P329, A330, P331 of SEQ ID NO:1 according to EU numbering.

According to an alternative example, the Fc region is aglycosylated.

More specifically, the amino acid substitutions in the Fc region are at positions L234 and L235, specifically L234A and L235A of SEQ ID NO:1 according to EU numbering.

Specifically provided herein is an Fc silenced anti-oxMIF antibody comprising an Fc region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:4, 5, 6, 7, 8, 9,43, and 85.

More specifically, the Fc-silenced anti-oxMIF antibody described herein comprises the variable heavy and light chains

i.) SEQ ID NOs:3 and 6,

ii.) SEQ ID NOs:9 and 6,

iii.) SEQ ID NOs:4 and 6,

iv.) SEQ ID NOs:4 and 8,

v.) SEQ ID NOs:4 and 5, or

vi.) SEQ ID NOs:43 and any one of 5, 6, 7 or 8.

According to an example, the Fc-silenced anti-oxMIF antibody described herein further comprises CH1 to CH3 domains of SEQ ID NO:14.

According to a specific example of the disclosure, the anti-oxMIF antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:15 and 16 or 17.

According to a further example, herein provided is an Fc silenced anti-oxMIF antibody or an antigen binding fragment thereof, comprising a variant Fc region of a wild-type human IgG comprising SEQ ID NO:1 with one or more amino acid substitutions or glycosylation modifications, comprising

a light chain CDR1 sequence selected from SEQ ID NOs: 72 or 78, and
a light chain CDR2 sequence selected from SEQ ID NOs: 73, 79, 80, or 81, and
a light chain CDR3 sequence selected from SEQ ID NOs: 74 or 82, and
a heavy chain CDR1 sequence selected from SEQ ID NOs: 75 and
a heavy chain CDR2 sequence selected from SEQ ID NOs: 76 or 83, and
a heavy chain CDR3 sequence selected from SEQ ID NOs: 77 or 84, with the provision that SEQ ID 74 and SEQ ID 77 are not comprised together.

In an alternative example, the Fc silenced anti-oxMIF antibody comprises
a light chain CDR1 sequence selected from SEQ ID NOs: 72 or 78, and
a light chain CDR2 sequence selected from SEQ ID NOs: 73, 79, 80, or 81, and
a light chain CDR3 sequence selected from SEQ ID NOs: 82, and
a heavy chain CDR1 sequence selected from SEQ ID NOs: 75 and
a heavy chain CDR2 sequence selected from SEQ ID NOs: 76 or 83, and
a heavy chain CDR3 sequence selected from SEQ ID NOs: 77 or 84.

In an alternative example, the Fc silenced anti-oxMIF antibody comprises

a light chain CDR1 sequence selected from SEQ ID NOs: 72 or 78, and

a light chain CDR2 sequence selected from SEQ ID NOs: 73, 79, 80, or 81, and

a light chain CDR3 sequence selected from SEQ ID NOs: 82, and

a heavy chain CDR1 sequence selected from SEQ ID NOs: 75 and

a heavy chain CDR2 sequence selected from SEQ ID NOs: 76 or 83, and

a heavy chain CDR3 sequence selected from SEQ ID NOs: 84.

In an alternative example, the Fc silenced anti-oxMIF antibody comprises

a light chain CDR1 sequence selected from SEQ ID NOs: 72 or 78, and

a light chain CDR2 sequence selected from SEQ ID NOs: 73, 79, 80, or 81, and

a light chain CDR3 sequence selected from SEQ ID NO: 82 and

a heavy chain CDR1 sequence selected from SEQ ID NOs: 75 or 83, and

a heavy chain CDR2 sequence selected from SEQ ID NOs: 76 or 84, and

a heavy chain CDR3 sequence selected from SEQ ID NO: 85

According to a further example of the disclosure, the Fc silenced anti-oxMIF antibody described herein has amino acid substitutions at any one of positions E233,

L234, L235, G236, G237, P238, I253, D265, S267, H268, N297, S298, T299, H310, E318, L328, P329, A330, P331, H435 of SEQ ID NO:1, specifically the amino acid substitutions are at positions L234 and L235, specifically at positions L234, L235, H310 and H435, according to EU numbering index, and/or an Fc region which is aglycosylated.

The Fc-silenced anti-oxMIF antibody described herein can be of any format which contains CH2 and CH3 antibody domains, specifically it is selected from the group consisting of monospecific, bispecific antibodies (e.g., Crossmab), scFv-Fc, $(scFv)_2$-Fc (two scFv fragments are comprised on one arm), scFv/scFv-Fc (i.e. each arm comprises an scFv fragment), Fab/scFv-Fc, Fab/$(scFv)_2$-Fc, Fab/Fab-scFv-Fc, Fab/Fab-crossFab-Fc, Fab/crossFab-Fc, IgG-scFv and IgG-$(scFv)_2$.

In a further example, the Fc-silenced anti-oxMIF antibody described herein is a bispecific antibody further comprising at least one binding site specifically recognizing an epitope of CD3, or histamine-succinyl-glycine (HSG).In a specific example, the Fc-silenced anti-oxMIF antibody is for use in the treatment or detection of solid tumors, wherein said antibody is administered to a subject in a first step and a HSG hapten is administered in a second step, wherein said HSG hapten binds to the antibody and is labelled with a radionuclide.

Herein provided in another aspect is also the Fc-silenced antibody described herein for use in the preparation of a medicament.

In yet a further aspect of the disclosure, herein provided is also a pharmaceutical composition comprising the antibody described herein, optionally together with a pharmaceutical carrier or adjuvant.

Specifically, the composition comprises 10 to 250 mg/ml of the inventive antibody, specifically comprising > 50 mg/ml.

More specifically, said pharmaceutical composition is formulated for subcutaneous administration.

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

In a further example provided herein is the pharmaceutical composition for use in the treatment of a patient suffering from inflammatory disease, infectious

disease, 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, NASH (nonalcoholic steatosis hepatitis), multiple sclerosis, acute and chronic pancreatitis, Type 1 diabetes, IgA nephropathy, interstitial cystitis, post COVID syndrome and psoriasis.

According to an alternative example, the pharmaceutical composition is for use in the treatment of a patient suffering from hyperproliferative disorder or cancer, specifically in the treatment of colorectal cancer, ovarian cancer, breast cancer, prostate cancer, pancreas cancer, gastric cancer, and lung cancer.

In one aspect, provided herein is the pharmaceutical composition described herein for use in the treatment of a patient suffering from inflammatory disease, infectious disease, 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, NASH (nonalcoholic steatosis hepatitis), multiple sclerosis, acute and chronic pancreatitis, Type 1 diabetes, IgA nephropathy, interstitial cystitis, post COVID syndrome and psoriasis, or for use in the treatment of a patient suffering from hyperproliferative disorder or cancer, specifically in the treatment of colorectal cancer, ovarian cancer, breast cancer, prostate cancer, pancreas cancer, gastric cancer, and lung cancer. In another aspect, provided herein is use of the pharmaceutical composition described herein in the manufacture of a medicament for the treatment of a patient suffering from inflammatory disease, infectious disease, 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, NASH (nonalcoholic steatosis hepatitis), multiple sclerosis, acute and chronic pancreatitis, Type 1 diabetes, IgA nephropathy, interstitial cystitis, post COVID syndrome and psoriasis, or for the treatment of a patient suffering from hyperproliferative disorder or cancer, specifically for the treatment of colorectal cancer, ovarian cancer, breast cancer, prostate cancer, pancreas cancer, gastric cancer, and lung cancer.

In one aspect, further provided herein is an isolated nucleic acid encoding the antibody of the disclosure.

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

In a further example, 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 disclosure, comprising culturing a host cell and recovering said antibody from the cell culture.

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

FIGURES

Figure 1: Chromatography profiles demonstrating reduced aggregation and hydrophobicity of the newly designed anti-oxMIF antibodies. (A) Comparison of elution profiles of C0008 (control antibody, gray area) and the parent antibodies (C0083 and C0090, without Fc silencing) of the newly designed antibodies C0115 and C0118 on a Enrich 650 gel filtration column using 1xPBS as mobile phase; (B) Comparison of elution profiles of C0008 (control antibody, gray area) and newly designed antibodies C0115 and C0118 on a Enrich 650 gel filtration column using 1xPBS as mobile phase (C) Comparison of elution profiles of C0008 (control antibody, gray area) and newly designed antibodies C0115 and C0118 and their parent antibodies C0083 and C0090 (without Fc silencing) on a HiTrap Butyl HP HIC column.

Figure 2: Binding curves of newly designed anti-oxMIF antibodies to immobilized oxMIF (K_D determination). Anti-oxMIF antibodies were detected by an anti-human-IgG (Fc)-