reducing aggregation of variable domains, e.g., rational design of aggregation-resistant proteins, complementarity determining region (CDR) grafting, introducing disulfide bonds into a variable domain or removing surface exposed hydrophobic patches. However, these methods show disadvantages, as they often alter binding properties such as affinity or specificity, are not generally applicable as they require specialized knowledge, are often laborious and might introduce immunogenic epitopes.

Further intrinsic properties of proteins such as hydrophobicity also play important roles in antibody solubility. Low solubility of these therapeutic proteins due to surface hydrophobicity may render formulation development more difficult and may lead to poor bio-distribution, undesirable pharmacokinetics behavior and immunogenicity *in vivo*. Decreasing the overall surface hydrophobicity of candidate monoclonal antibodies could thus provide benefits and cost savings relating to purification and dosing regimens.

Although increasing efforts are made in RIT and radio-imaging and despite the growing number of monoclonal antibodies used in clinical trials, there is a continuous need for improved radioimmunotherapy and imaging providing efficient therapeutic effects in cancer treatment and diagnosis, specifically involving antibodies with highly selective binding to tumor associated antigens.

SUMMARY

It is the objective of the present disclosure to provide a radioimmunoconjugate with highly selective tumor-binding properties.

The objective is solved by the subject matter of the present disclosure. Although oxMIF seems to fulfill the requirements as tumor specific target, several facts did not make oxMIF an obvious target for radioimmunotherapy. OxMIF is not precisely characterized, the molecular mechanism of disease related generation of oxMIF is still unclear and oxMIF is not considered a cell surface receptor.

In spite of this, it has been surprisingly shown that a radiolabeled anti-oxMIF antibody or antigen binding fragment thereof (anti-oxMIF radioimmunoconjugate) is highly selective due to its oxMIF-specificity and is highly cytotoxic due to the presence of a radioisotope, when used in radio-immunotherapy.

The radioimmunoconjugate can also be readily detected in tumor tissue due to its oxMIF-specificity and the appropriate radionuclide, when used in radio-imaging.

The radioimmunoconjugate is also highly selective due to its oxMIF-specificity and allows diagnosis and therapy due to the radionuclide, when used in a theranostic

approach. The combination of a radionuclide and an anti-oxMIF antibody has surprisingly shown that the radiolabeled anti-oxMIF antibody has a relevant biodistribution, tumor uptake and efficacy, already proven in a mouse model. Therefore, the inventive radioimmunoconjugate is highly efficient for cancer therapy and also highly valuable for tumor detection and diagnosis.

The present disclosure discloses a radioimmunoconjugate (RIC) comprising a recombinant anti-oxMIF antibody or an antigen binding fragment thereof.

In one aspect, the present disclosure specifically provides a radioimmunoconjugate (RIC) comprising a recombinant anti-oxMIF antibody or an antigen binding fragment thereof, comprising:

(a1) a light chain variable domain comprising SEQ ID NO:32 having at least one of amino acid substitutions M30L, F49Y, A51G, P80S, W93F, or

(a2) a light chain variable domain comprising SEQ ID NO:32 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:29; or

(b2) a heavy chain variable domain comprising SEQ ID NO:29 and the amino acid substitutions L5Q and/or W97Y, or

(b3) a heavy chain variable domain comprising SEQ ID NO:29 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 said antibody or antigen binding fragment thereof has reduced aggregation potential and reduced hydrophobicity compared to an antibody or an antigen binding fragment thereof comprising SEQ ID NO:32 and SEQ ID NO:29 lacking the amino acid substitutions.

The related germline VL sequences of the anti-oxMIF antibody can have a high variability at positions D1, M4, S10, and L11 of framework 1.

According to a specific example, the anti-oxMIF antibody therefore can further comprise a light chain variable domain comprising SEQ ID NO:32 with 1, 2, 3, 4, or 5 amino acid substitutions in addition to further substitutions at positions D1, M4, S10, and L11.

The related germline VH sequences can have a high variability at the following positions: E1, S74 and T77.

According to a further specific example, the anti-oxMIF antibody therefore can further comprise a heavy chain variable domain comprising SEQ ID NO:29 with 1, 2, 3, 4, or 5 amino acid substitutions in addition to further substitutions at positions E1, S74 and T77.

In an alternative example of the disclosure, herein provided is a radioimmunoconjugate (RIC) comprising a recombinant anti-oxMIF antibody or an antigen binding fragment thereof, comprising:

(a) an anti-oxMIF antibody light chain variable domain, comprising a light chain variable domain with at least 95% sequence identity to SEQ ID NO:32, comprising a tyrosine at position 36 and further comprising at least one of amino acid substitutions M30L, F49Y, A51G, P80S, W93F, and

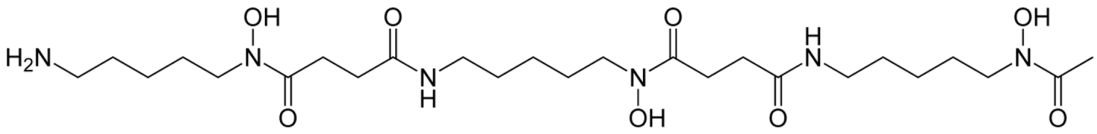
(b) a heavy chain variable domain comprising SEQ ID NO:29 and amino acid substitution W97Y, or a heavy chain variable domain comprising at least 95% sequence identity to SEQ ID NO:29 and further comprising amino acid substitution W97Y.

According to yet a further example, the radioimmunoconjugate contains a light chain variable domain with the amino acid substitution W93F and a heavy chain variable region comprising the amino acid substitution W97Y.

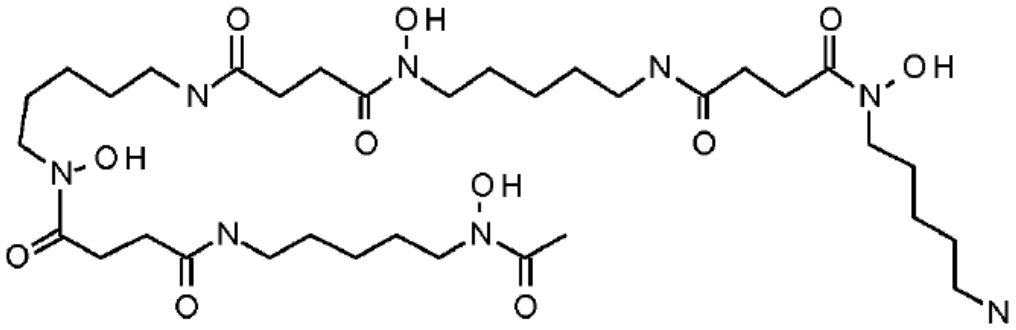
According to a specific example of the disclosure, the anti-oxMIF antibody or an antigen binding fragment thereof is directly labelled or labelled using acyclic, monocyclic, macrocyclic bifunctional chelating agents, in particular a chelating agent selected from p-SCN-bn-DOTA, p-NH2-Bn-DOTA, DOTA-NHS and p-SCN-Bn-deferoxamine comprising a chelating group and a functional/reactive linker.

According to a specific example, the radioimmunoconjugate comprises a chelating group, specifically a chelating group resulting from coupling the anti-oxMIF antibody or an antigen binding fragment thereof to a chelating agent, in particular a chelating group selected from DOTA, a hexadentate chelator such as deferoxamine B (CAS No. 70-51-9), and an octadentate chelator such as the DFO derivative DFO* or an oxygen containing analogue thereof, such as oxoDFO*.

Specifically, the DFO can be of following structure:



Specifically, DFO* can be of following structure:



According to the disclosure, any alpha- or beta-emitting radioisotope useful in therapeutic application can be linked to the anti-oxMIF antibody, specifically it can be ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{32}P, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{89}Zr, ^{90}Y, ^{99}mTc, ^{103}Pd, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{140}La, ^{149}Tb, ^{149}Pm, ^{153}Sm, ^{159}Gd, ^{165}Dy, ^{166}Dy, ^{166}Ho, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{227}Th, ^{186}Re, ^{188}Re, ^{192}Ir, ^{193}mPt, ^{195}mPt, ^{198}Au, ^{199}Au, ^{211}At, ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{225}Ac, ^{223}Ra, ^{227}Th, specifically the radioisotope is ^{67}Ga, ^{89}Zr, ^{111}In, ^{124}I, ^{131}I, ^{177}Lu, ^{225}Ac.

In a specific example there is provided a radioimmunoconjugate as described herein, comprising a Fc variant domain of a wild-type human IgG1 constant domain having SEQ ID NO: 37, comprising at least one amino acid substitution at any one of positions L234, L235, G236, G237, N297, L328, or P329, and wherein said radioimmunoconjugate exhibits decreased binding to Fcγ-receptors compared to a radioimmunoconjugate comprising the wildtype IgG1 Fc region.

In a further specific example, the radioimmunoconjugate comprises an Fc variant domain of a wild-type human IgG1 constant domain of SEQ ID NO:37, comprising one, two or three amino acid substitutions at any one of positions, I253, H310, H435, and wherein said radioimmunoconjugate exhibits reduced affinity to the human FcRn compared to a radioimmunoconjugate comprising the wildtype IgG1 Fc region.

Specifically, the radioimmunoconjugate comprises SEQ ID NO:38 and exhibits reduced affinity to the human FcRn compared to a radioimmunoconjugate comprising the wildtype IgG1 Fc region.

In a specific example, the radioimmunoconjugate comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:31, 33, 34, 35, and 36.

In a further specific example, the radioimmunoconjugate comprises SEQ ID NOs:29 and 34; SEQ ID NOs:30 and 33, SEQ ID NOs:30 and 34, SEQ ID NOs:31 and 34, SEQ ID NOs:31 and 36, or SEQ ID NOs:31 and 33, in combination with any one of SEQ ID NOs:37, 38, 39, and 40.

More specifically, the radioimmunoconjugate described herein can be, but is not limited to an IgG, IgG-fusion protein, Fv, scFv, scFv-fusion protein, diabody, diabody fusion protein, Fab, Fab-fusion protein, scFab, scFab-fusion protein, Fab', and F(ab')2, Fab'-SH, Fcab, Fcab fusion protein, a fusion protein of two or more single chain antibodies, minibody, and small immune protein (SIP) format.

According to an aspect, the radioimmunoconjugate described herein is for use in the preparation of a medicament.

In a specific example, a pharmaceutical composition is provided herein, comprising the radioimmunoconjugate described herein, optionally together with a pharmaceutical carrier or adjuvant.

Specifically, the pharmaceutical composition is formulated for intravenous administration.

In one aspect, the present disclosure provides a pharmaceutical composition comprising the radioimmunoconjugate described herein, optionally together with a pharmaceutical carrier or adjuvant, specifically formulated for intravenous administration.

According to a further example, the pharmaceutical composition is for use in the treatment of a subject suffering from cancer, specifically in the treatment of tumors, specifically of hematological or solid tumors, more specifically for the treatment of colorectal cancer, ovarian cancer, breast cancer, brain tumors, prostate cancer, pancreas cancer, and lung cancer. In one aspect, the present disclosure provides use of the pharmaceutical composition described herein or the radioimmunoconjugate described herein in the manufacture of a medicament for the treatment of a patient suffering from cancer, specifically in the treatment of tumors, specifically of hematological or solid tumors, more specifically for the treatment of colorectal cancer, ovarian cancer, breast cancer, prostate cancer, pancreas cancer, and lung cancer.

An example of the present disclosure relates to the use of an effective amount of the pharmaceutical composition of the present disclosure in a method for treatment of any one of the diseases or symptoms listed herein.

An example of the present disclosure relates to the use of the radioimmunoconjugate of the present disclosure in combination with or in addition to other therapy.

In an example of the present disclosure the other therapy is selected from chemotherapy, monoclonal antibody therapy, surgery, radiotherapy, and/or photodynamic therapy.

According to an example, the radioimmunoconjugate described herein is used for the detection or determination of the oxMIF level in the cancer cells of a subject, specifically detection is performed *ex vivo* in a sample of the subject. In one aspect, the present disclosure provides use of the radioimmunoconjugate described herein for detection of oxMIF level in the cancer of a subject. Potential patient groups may then be divided into subgroups (strata, blocks) by stratification according to the oxMIF level, wherein each strata represents a particular section of the patient population.

Specifically, the radioimmunoconjugate is used for cancer diagnosis in a subject. In one aspect, the present disclosure provides use of the radioimmunoconjugate described herein for diagnosis of cancer in a subject.

In an example herein provided is a method for in vitro diagnosing cancer, wherein the radioimmunoconjugate is used for in vitro detecting tumor cells in a sample of a subject or for in vivo detecting tumor cells or tumors in a subject. In one aspect, the present disclosure provides a method for in vivo diagnosing cancer in a subject, wherein the radioimmunoconjugate described herein is used for detecting tumor cells. In one aspect, the present disclosure provides a method for in vitro diagnosing cancer, wherein the radioimmunoconjugate described herein is used for detecting tumor cells in a sample. Said sample can be for example a blood sample, a tissue sample, e.g. from biopsy.

Further provided herein is a method for diagnosing cancer, wherein the radioimmunoconjugate is used for detecting tumor cells in a subject or, in vitro, in a sample of the subject.

A further example relates to a diagnostic kit comprising the radioimmunoconjugate described herein.

In an alternative example, a kit for production of the radioimmunoconjugate is provided, comprising two or more vials, wherein one vial contains a conjugate comprising the chelator linked to an anti-ox-MIF antibody and a second vial contains a radioisotope, specifically selected from ^{67}Ga, ^{89}Zr, ^{111}In, ^{124}I, ^{131}I, ^{177}Lu, ^{225}Ac. Specifically, the content of one or several of the vials is lyophilized or in solution. In one aspect, the present disclosure refers to a kit for production of the radioimmunoconjugate described herein, comprising two or more vials, wherein one vial contains a conjugate comprising a chelator linked to an anti-oxMIF antibody and a second vial contains a radioisotope, specifically selected from ^{177}Lu and ^{89}Zr, and wherein the content of one or several of the vials is optionally lyophilized or in solution. More specifically, the kit further comprises a manual containing instructions to prepare the radioimmunoconjugate described herein.

Further provided herein is a method for treating cancer using the radioimmunoconjugate described herein or a pharmaceutical composition comprising said radioimmunoconjugate. In one aspect, the present disclosure provides use of the radioimmunoconjugate described herein, or a pharmaceutical composition described herein, in the manufacture of a medicament for treating cancer.

Further provided in another aspect is an isolated nucleic acid encoding the recombinant anti-oxMIF antibody or antigen binding fragment thereof of the radioimmunoconjugate described herein.

In one aspect, there is provided an expression vector comprising the nucleic acid described herein. In a further example, an expression vector comprising the nucleic acid molecule(s) encoding the recombinant anti-oxMIF antibody or antigen binding fragment thereof of the radioimmunoconjugate.

In one aspect, there is provided a host cell containing the nucleic acid described herein or an expression vector described herein. In a further example there is provided a host cell containing the nucleic acid of the oxMIF antibody described herein or an expression vector comprising the nucleic acid molecule(s) described herein.

In yet a further example, a method for producing the radioimmunoconjugate is provided, comprising expressing a nucleic acid encoding an oxMIF antibody or antigen binding fragment in a host cell and further labeling said antibody or antigen binding fragment with a radioisotope. In one aspect, there is provided a method for producing the radioimmunoconjugate described herein, comprising expressing a nucleic acid encoding an anti-oxMIF antibody in a host cell and further labeling said antibody with a radioisotope.

FIGURES

Figure 1: Purity and integrity analysis of DOTA-C0008; A: DOTA-C0008 SE-HPLC profile (λ=280 nm). B: Coomassie stained SDS PAGE: 1: molecular weight standard; 2: C0008 non-reduced; 3: C0008 reduced; 4: Concentrated C0008 (labeling batch) non-reduced; 5: Concentrated C0008 (labeling batch) reduced; 6: DOTA-C0008 non-reduced; 7: DOTA-C0008 reduced.

Figure 2: Determination of the number of DOTA molecules grafted to C0008 determined by ITLC: ITLC separation of DOTA-C0008 which was incubated with a mixture of tracer amounts of radioactive indium (^{111}In) and stable indium (^{115}In) at 2 (lane 6), 4 (lane 7), 6 (lane 8) or 8 (lane 9) fold excess of $^{111/115}$In to the DOTA conjugated antibody.

Figure 3: Analysis of radiolabeled C0008 by ITLC and Autoradiography: A: ITLC of radiolabeled C0008 (Lane 3: ^{177}Lu labelling reaction with DOTA-C0008; Lane 4: ^{177}Lu labelling reaction with C0008); B: Autoradiogram of ITLC film lane 3 (^{177}Lu-DOTA-C0008 after DTPA addition; Peak 1 reflects free ^{177}Lu-DTPA and Peak 2 reflects ^{177}Lu-DOTA-C0008).

Figure 4: Plasma stability of ^{177}Lu-DOTA-C0008 (autoradiography): Autoradiogram of ^{177}Lu-DOTA-C0008 incubated in mouse plasma for 7 days; 1: ^{177}Lu-DOTA-C0008 derivatives and free ^{177}Lu; 2: ^{177}Lu-C0008.

Figure 5: Plasma stability of ^{177}Lu-DOTA-C0008 (SDS-PAGE): Reduced SDS PAGE with ^{177}Lu-DOTA-C0008 in mouse plasma (left: Coomassie staining; right: Autoradiography). Lane 1 and Lane 10: molecular weight standards; Lane 2: DOTA-C0008; Lane 3: ^{177}Lu-DOTA-C0008 in buffer (T0); Lane 5: ^{177}Lu-DOTA-C0008 (T0 hours