

Richter et al. (2013, PLoS One 8:e72156) describe the inhibition of TNFR1 to interact with its natural ligands TNF and lymphotoxin alpha (LT α) by ATROSAB as measured by the release of IL-6 and IL-8 from HeLa and HT1080 cells, respectively, induced by TNF or LT α .

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SUMMARY

It was the objective to provide an improved anti-huTNFR1 agent with improved TNFR1-inhibiting characteristics while avoiding any side effects caused by intrinsic
10 TNF mimetic agonistic activity.

The object is solved by the subject matter as claimed.

In one aspect of the present disclosure, there is provided an inhibitor of the huTNFR1 receptor which is a human or humanized antibody construct that monovalently recognizes huTNFR1 through an antigen-binding moiety, wherein the
15 antigen-binding moiety comprises

- a heavy chain variable (VH) domain that comprises the CDR sequences CDRH1, CDRH2, and CDRH2, and
- a light chain variable (VL) domain that comprises the CDR sequences CDRL1, CDRL2, and CDRL2, wherein:

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- a) the CDRH1 sequence is identified as SEQ ID 1;
- b) the CDRH2 sequence is identified as SEQ ID 10;
- c) the CDRH3 sequence is identified as SEQ ID 3;
- d) the CDRL1 sequence is identified as SEQ ID 4;
- e) the CDRL2 sequence is identified as SEQ ID 5; and
- f) the CDRL3 sequence is identified as SEQ ID 11;

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or

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- a) the CDRH1 sequence is a functionally active CDR variant of SEQ ID 1;

30 and/or

- b) the CDRH2 sequence is a functionally active CDR variant of SEQ ID 10;

and/or

c) the CDRH3 sequence is a functionally active CDR variant of SEQ ID 3;
and/or

d) the CDRL1 sequence is a functionally active CDR variant of SEQ ID 4; and/or

5 e) the CDRL2 sequence is a functionally active CDR variant of SEQ ID 5; and/or

f) the CDRL3 sequence is a functionally active CDR variant of SEQ ID 11;

wherein the functionally active CDR variant comprises not more than 1 or 2 point mutations in the respective CDR sequence at any position, except at position 5 in CDRH2 and at position 3 in CDRL3.

10 In another aspect of the present disclosure, there is provided a pharmaceutical preparation comprising the inhibitor disclosed herein and a pharmaceutically acceptable carrier, preferably wherein the preparation is formulated for parenteral use, preferably by intravenous or subcutaneous administration.

15 In a further aspect of the present disclosure, there is provided a method of producing an inhibitor disclosed herein employing a recombinant mammalian expression system to express the antibody construct, preferably wherein a CHO production cell line is employed.

20 In another aspect of the present disclosure, there is provided an inhibitor disclosed herein, for use in treating a human subject suffering from a disease where anti-TNF therapies or non-biologic disease-modifying anti-rheumatic drugs (DMARD) are indicated, preferably as first line treatment, or as second line treatment where anti-TNF or non-biologic DMARD therapeutics failed.

25 In a further aspect of the present disclosure, there is provided a use of an inhibitor disclosed herein in the manufacture of a medicament for treating a human subject in need of an anti-TNF therapy.

In another aspect of the present disclosure, there is provided an isolated nucleic acid encoding the inhibitor disclosed herein.

In a further aspect of the present disclosure, there is provided an expression vector comprising the nucleic acid disclosed herein.

In another aspect of the present disclosure, there is provided a recombinant host cell comprising the nucleic acid disclosed herein or the expression vector disclosed herein.

5 According to the present disclosure there is provided an inhibitor of the TNF - huTNFR1 receptor interaction which is a human or humanized antibody construct that monovalently recognizes huTNFR1 through an antigen-binding moiety.

Specifically, the antigen-binding moiety comprises

- a heavy chain variable (VH) domain that comprises the CDR sequences
10 CDRH1, CDRH2, and CDRH2, and
- a light chain variable (VL) domain that comprises the CDR sequences CDRL1, CDRL2, and CDRL2, wherein

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- a) the CDRH1 sequence is identified as SEQ ID 1;
- 15 b) the CDRH2 sequence is identified as SEQ ID 10;
- c) the CDRH3 sequence is identified as SEQ ID 3;
- d) the CDRL1 sequence is identified as SEQ ID 4;
- e) the CDRL2 sequence is identified as SEQ ID 5; and
- f) the CDRL3 sequence is identified as SEQ ID 11;

20 or

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- a) the CDRH1 sequence is a functionally active CDR variant of SEQ ID 1;
- and/or
- b) the CDRH2 sequence is a functionally active CDR variant of SEQ ID 10;
- 25 and/or
- c) the CDRH3 sequence is a functionally active CDR variant of SEQ ID 3;
- and/or

the range of 50-100 Hz, e.g. at about 50 Hz, or at 50 Hz +/- 10 Hz, or at 50 Hz +/- 5 Hz. A standard test for determining the affinity of binding by QCM is described in the examples section below.

Specifically, K_D is less than 4×10^{-9} M, or less than 3×10^{-9} M, or less than 2×10^{-9} M, or less than 10^{-9} M, or even less than 10^{-10} M

Specifically, the k_{off} is less than 10^{-3} , or less than $5 \times 10^{-4} \text{ s}^{-1}$, or less than 10^{-4} s^{-1} , or less than 10^{-5} s^{-1} .

Specifically, the antigen-binding moiety is recognizing the huTNFR1 with a k_{on} of at least $10^5 \text{ M}^{-1} \text{ s}^{-1}$.

According to a specific embodiment, the inhibitor directly inhibits the TNF - huTNFR1 receptor interaction as determined in a cell-based assay, preferably by an assay for inhibition of TNFR1-mediated cell death in Kym-1 cells, or by an assay for inhibition of IL-6 or IL-8 release from HeLa cells or HT1080 cells, respectively. Specifically, in an assay for inhibition of TNFR1-mediated cell death in Kym-1 cells the IC_{50} value is less than 5.0×10^{-9} M. Specifically, in an assay for inhibition of IL-6 release from HeLa cells the IC_{50} value is less than 4.0×10^{-8} M, or in an assay for inhibition of IL-8 release from HT1080 cells the IC_{50} value is less than 2.0×10^{-8} M.

Due to monovalent interaction, a TNF-mimetic agonistic potential of the antibody construct has been specifically eliminated, while the increased affinity of binding to TNFR1, specifically the low off rate, provides superior inhibition of TNFR1-dependent TNF responses.

According to a further specific embodiment, the inhibitor directly inhibits the huTNFR1 - receptor interaction with lymphotoxin alpha as determined in a cell-based assay, preferably by an assay for inhibition of TNFR1-mediated cell death in Kym-1 cells, or by an assay for inhibition of IL-6 or IL-8 release from HeLa cells and/or HT1080 cells.

Specifically, the antigen-binding moiety comprises or consists of one or more Fv domains which form the monovalent binding site. According to a specific embodiment, the Fv domains are a VH and a VL domain, both in association with each other through interaction of the beta-sheet structure of the domains.

According to one embodiment, the antigen-binding moiety is specifically recognizing an epitope comprising the membrane-distal CRD1 and subdomain A1 of CRD2 of huTNFR1.

A specific binding epitope is represented by amino acid 1 to 115 in the N-terminal region of huTNFR1.

According to a specific embodiment, the antibody construct is selected from the group consisting of Fab molecules, scFv molecules, single variable domains, disulfide-stabilized Fv (dsFv), half-IgG1 antibodies, and Fv domains, or a functionally active derivative of any of the foregoing, preferably wherein the antibody construct is coupled to a hydrophilic polymer, such as PEG, and/or fused to a polypeptide, such as human (or mouse) serum albumin, transferrin, albumin-binding domains or peptides, Ig-binding domains or peptides, PEG-mimetic polypeptide extensions, an antibody Fc fragment, an antibody Fc fragment carrying mutations to allow for preferred heterodimerization (over homodimerization), or a functional variant of any of the foregoing polypeptides.

Specifically, the antibody construct is any of a Fab, scFv, dsFv, or Fv domains, which is fused to an antibody Fc fragment, wherein the Fc consists of a heterodimer of CH2 and CH3 domains, wherein the CH2 and/or CH3 domains carry one or more point mutations which allow preferential heterodimerization over homodimerization. Specifically, one or both of the CH3 domains in the Fc are modified to change the amino acid structure, such as to obtain a Fc containing the heterodimer of the CH3/CH3 domains.

Specifically, the antibody construct comprises Fv domains fused to an antibody Fc region or fragment, with or without further antibody domains, yet, maintaining the monovalent binding structure of the antibody construct. A specific example refers to a Fab moiety or Fv moiety fused to Fc or modified Fc.

Specific embodiments comprise a human IgG1 Fc wherein the CH2-CH3 domains form a heterodimer through one or more “knobs-into-holes” mutations, e.g.

“knobs” mutations modifying the surface of CH3 beta-sheets, present on one CH3 domain monomer, which is T366W; and

“holes” mutations modifying the surface of CH3 beta-sheets, present on the other CH3 domain monomer, which are selected from the group consisting of T366S, L368A, Y407V.

Specifically, the antibody construct comprises an Fc region which comprises one or more mutations to downmodulate the effector function. According to a specific embodiment, the Fc region is glycoengineered to downmodulate the effector function.

Specifically, the antibody construct comprises a (human IgG1) Fc region which is characterized by FcγR silenced CH2 and CH3 domains, e.g., through one or more mutations selected from the group consisting of E233P, L234V, L235A, ΔG236,

GTDKTHTSPPCPAPPVAG (SEQ ID 42), GTDKTHTCPPSPAPPVAG (SEQ ID 43), or GTDKTHTSPPSPAPPVAG (SEQ ID 44).

Specifically, the antibody construct is a disulfide-stabilized Fv (dsFv), which is characterized by one or more additional (artificial) interdomain disulfide bonds. Such
5 disulphide bonds are obtained by introducing one or more additional cysteine residues into either of the VH and VL domains at suitable positions which may be used as a bridge pier of disulphide bonds bridging the VH and VL domains, which disulphide bonds are obtained upon reducing the cysteines. According to specific examples, a disulphide bond may be introduced into the Fv at any of the following positions in VH
10 and corresponding positions in VL: 44C in VH and 100C in VL, 108C in VH and 55C in VL, 106C in VH and 56C in VL, or 101C in VH and 46C in VL.

According to a specific embodiment, the antibody construct comprises Fv domains with increased affinity to bind the huTNFR1 as compared to parent Fv domains wherein the parent Fv domains are characterized by a parent VH domain
15 identified as SEQ ID 12 and a parent VL domain identified as SEQ ID 16.

Specifically, at least one of the VH and VL domains is an affinity-matured functional variant of the parent domain, comprising at least one point mutation in any of the CDR or framework (FR) sequences.

Specifically,

20 a) the VH domain comprises or consists of a sequence selected from the group consisting of SEQ ID 13-15, or a functionally active variant of any of SEQ ID 13-15; and/or;

b) the VL domain comprises or consists of a sequence selected from the group consisting of SEQ ID 17-19, , or a functionally active variant of any of SEQ ID 17-19; or

25 c) wherein the functionally active variant of a) or b) comprises at least one point mutation in any of the CDR or FR sequences. Specifically, the functionally active variant of a) or b) is characterized by

a) 1 or 2 point mutations in any of the CDR sequences at any position except at position 5 in CDRH2 and at position 3 in CDRL3; and/or

30 b) at least one point mutation in the framework region of any of the VH or VL sequences.

Specifically, the inhibitor comprises a combination of a VH and a VL domain, which is

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a combination of a VH and VL domain of any of the group members i) – ix) of A, wherein the VH domain is a functionally active variant of any of SEQ ID 13-15, and/or the VL domain is a functionally active variant of any of SEQ ID 17-19, which functionally active variant is characterized by

a) 1 or 2 point mutations in any of the CDR sequences at any position except at position 5 in CDRH2 and position 3 in CDRL3; and/or

b) at least one point mutation in the framework region of any of the VH or VL sequences.

The functionally active variant may be a functionally active CDR variant with at least one point mutation in any of the CDR sequences, and/or a functionally active variant with at least one point mutation in any of the FR sequences. Yet, the functionally active CDR variants specifically are characterized by the CDRH2 sequence, wherein the amino acid sequence at position 5 is any of G or S; and the CDRL3 sequence, wherein the amino acid sequence at position 3 is any of G or S. The functionally active CDR variant specifically is characterized by the high affinity of binding the huTNFR1, such as further described herein.

According to a specific embodiment, the antibody construct has an increased thermostability as compared to a parent antibody construct wherein the antibody construct comprises Fv domains which are functional variants of parent Fv domains with at least one point mutation in the framework region of any of the VH or VL sequences, preferably wherein the VH domain sequence is a variation of the (parent) IZI06.1 VH sequence (SEQ ID 20) and comprises any of the amino acids (Kabat numbering):

a) in FR1 at position 1: Q or H;

b) in CDRH2

i) at position 3: Y or V;

ii) at position 5: Y, T, or S;

iii) at position 6: S or Q;

iv) at position 8: H or E;

v) at position 10: Y or K;

vi) at position 13: E or D.

A further preferred variation concerns the VL sequence (SEQ ID 21) and comprises the S91G point mutation in the CDRL3 (position 3).

Specifically, the thermostability of preferred variants of the antibody construct or the inhibitor is at least 60°C, or at least 61°C, or at least 62°C or at least 63°C, or at least 64°C, or at least 65°C, as determined by dynamic light scattering.

5 The present disclosure further provides for a pharmaceutical preparation comprising the inhibitor described herein and a pharmaceutically acceptable carrier.

Because of the antagonistic properties of the inhibitor and the antibody construct, the pharmaceutical preparation may comprise high concentrations of the inhibitor, while avoiding the side effects resulting from agonistic activity.

10 In the absence of the full-length immunoglobulin structure, the inhibitor specifically has reduced immunogenicity and may be repeatedly used without formation of inhibitors, such as anti-drug antibodies (ADA).

It has surprisingly turned out that the inhibitor can be used for treating patients developing ADA, e.g. which have developed antibodies against immunoglobulin or antibody immunotherapeutics. In the prior art, the presence of such ADA would particularly exclude further immunotherapies with antibodies directed against TNFR1, because ADA have the potential to cross-link the antibodies upon binding the TNFR1 on the cell surface, thereby agonising the TNFR1 signaling. However, the inhibitor described herein surprisingly does not agonise the TNFR1 signaling even in the presence of ADA.

20 Specifically, the pharmaceutical preparation may be administered to subjects who have developed ADA, e.g. ADA against anti-huTNFR1 antibodies or any IgG structures.

Specifically, the preparation is formulated for parenteral use, preferably by intravenous or subcutaneous administration.

25 The present disclosure further provides for a method of producing the huTNFR1 inhibitor described herein employing a recombinant mammalian expression system to express the antibody construct.

Specifically, production cell lines are used which are eukaryotic or mammalian cell lines, including cell lines of different origin e.g. human, such as HEK or PER.C6; hamster, such as CHO or BHK; monkey, such as COS-1 or COS-7; mouse, such as C127, SP2/0 or NS0; or yeast, such as *Saccharomyces cerevisiae* or *Pichia pastoris*.

Specifically, a CHO production cell line is employed.

Alternative prokaryotic production cell lines include e.g. *Escherichia coli*.

The present disclosure further provides for the inhibitor for medical use, specifically, for use in treating a human subject in need of an anti-TNF therapy.

Thus, the present disclosure further provides for a method of treating a human subject in need of an anti-TNF therapy, by administering an effective amount of the
5 inhibitor described herein.

Specifically, the TNFR1-specific inhibitor is used as a TNF antagonist incapable of crosslinking TNFR1, as an alternative to treatment with an anti-TNF therapeutic. Such TNF antagonists, also considered as biological TNF antagonists, are typically provided for therapeutic use where the biological relevance of TNFR1-mediated TNF
10 function in the pathogenesis of chronic noninfectious inflammation of joints, skin and gut has been proven.

Drug-specific antibodies (ADA) induced by therapeutic antibodies or naturally occurring antibodies may also lead to undesired agonistic activity, because of the potential to cross-link drug-bound TNFR1 and activate the TNFR1 signaling. Thus, the
15 preferred antibody construct is devoid of a homodimerizing Fc region or devoid of an Fc region, such as a Fab or scFv format, and the respective pharmaceutical composition is less likely to result in cross-linking and increased stimulatory activity to inflammatory processes.

According to a specific embodiment, the inhibitor is repeatedly administered to
20 the subject.

It is preferred that the inhibitor or antibody described herein has a low immunogenic potential and can be used for treating a subject without inducing undesired immune response to the antibody.

To this end, the preferred antibody construct consists of humanized or human
25 antibody sequences, and is e.g. devoid of a homodimerizing Fc region or devoid of an Fc region, such as a Fab or scFv format. The respective pharmaceutical composition is less likely to result in a loss of tolerance (e.g., upon repeated administration), thereby improving both the safety and efficacy profile of the therapeutic.

Specific embodiments refer to the treatment of subjects suffering from an
30 immune response or a high level of antibodies against therapeutic antibodies, in particular antibodies comprising an Fc region which have been used for previous therapies. Such patients are preferably treated with an antibody construct described herein devoid of the homodimerizing Fc region or devoid of the Fc region.

Specifically, the inhibitor described herein is provided for medical use in treating a human subject suffering from a disease where anti-TNF therapies or non-biologic disease-modifying anti-rheumatic drugs (DMARD) are indicated. Specifically, the medical use encompasses a first line treatment with an effective amount of the inhibitor, where anti-TNF therapies or non-biologic DMARD are indicated, or as second line treatment where anti-TNF or non-biologic DMARD therapeutics failed.

Specifically, the subject is suffering from

a) acute or chronic inflammation of joints, skin and gut, (infectious or non-infectious); and/or

b) autoimmune diseases, rheumatoid arthritis, psoriasis, psoriatic arthritis, juvenile arthritis, ankylosing spondylitis, Crohn's disease (Morbus Crohn), multiple sclerosis, congestive heart failure, metabolic disease, cytokine release syndrome, septic shock, acute and chronic neurodegenerative disease, stroke, Alzheimer and Parkinson disease, colitis ulcerosa, pancreatitis, COPD, acute fulminant viral or bacterial infections, metabolic diseases, chronic neurodegenerative diseases, genetically inherited diseases with TNF/TNFR1 as the causative pathologic mediator, periodic fever syndrome, Cherubism, and cancer.

In particular, inflammatory disease conditions are treated which are associated with any of the diseases listed above. Specifically, the subject is treated suffering from any of the diseases of a) and any of the diseases of b) above.

According to a specific embodiment, the present disclosure further provides for an isolated nucleic acid encoding the inhibitor described herein. Specifically, the nucleic acid is operably linked to a non-coding or coding nucleic acid sequence not naturally-occurring with the nucleic acid, such as including heterologous promoter or regulatory sequences. Specifically, the nucleic acid is an artificial nucleic acid.

The present disclosure further provides for a vector comprising an expression cassette or a plasmid, each comprising a coding sequence to express a proteinaceous construct comprising or consisting of a polypeptide or protein, or a protein derivative, comprising the antigen-binding site or the a VH and/or VL of the inhibitor antibody as described herein.

The present disclosure further provides for a host cell comprising the expression cassette, expression vector or plasmid as described herein.

The present disclosure further provides for a method of producing the inhibitor as described herein, wherein the host cell is cultivated or maintained under conditions to produce said antibody.

Specifically preferred is a host cell and a production method employing such host cell, which host cell comprises

- an expression vector, which incorporates a coding sequence to express the antibody light chain; and
- an expression vector, which incorporates a coding sequence to express the antibody heavy chain.

According to a specific embodiment, the present disclosure further provides for a recombinant host cell comprising the nucleic acid or the expression vector described herein.

FIGURES

Figure 1. Binding of H398 and ATROSAB to human TNFR1-Fc. a) Equilibrium binding of ATROSAB and H398 was analyzed by standard ELISA (n=3, mean + SD). QCM binding kinetics of ATROSAB (b) and H398 (c) tested under conditions of high receptor density (195 Hz). Applied were triplicates of five concentrations between 3.9 nM and 62.5 nM.

Figure 2. Inhibition of TNF action by ATROSAB and H398. Inhibition of IL-8 secretion from HT1080 cells with increasing concentrations of ATROSAB and H398 induced by 0.1 nM TNF (a) and LT α (b). Data from n = 3 experiments are shown as percent of maximal IL release, triggered by TNF or LT α alone.

Figure 3. Binding kinetics at low receptor density. Determination of the affinity of Atrosab (a) and H398 (b) for human TNFR1-Fc by QCM at a receptor density of 48 Hz.

Figure 4. Sequence alignment of scFvIZI06.1 (VH: SEQ ID 20, VL: SEQ ID 21) with parent VH and VL sequences and affinity matured variants. a) aligned VH sequences of ATROSAB (scFvIZI06.1), scFvIG11, scFvT12B and scFvFRK13.7. b) Alignment of VL sequences. Residues are numbered according to the Kabat numbering scheme and dots represent identical amino acids compared with scFvIZI06.1. Sequences of 4 amino acids or more are indicated as follows. Sequences in FR1 of scFvFRK13.7: SASV (SEQ ID 36); DRV T (SEQ ID 37). Sequence in FR3 of scFvFRK13.7: SLQP (SEQ ID 38).

11. A method of producing an inhibitor of any of claims 1 to 9 employing a recombinant mammalian expression system to express the antibody construct, preferably wherein a CHO production cell line is employed.

5 12. The inhibitor of any of claims 1 to 9, for use in treating a human subject suffering from a disease where anti-TNF therapies or non-biologic disease-modifying anti-rheumatic drugs (DMARD) are indicated, preferably as first line treatment, or as second line treatment where anti-TNF or non-biologic DMARD therapeutics failed.

10 13. The inhibitor for use according to claim 12, wherein the subject has developed anti-drug antibodies.

14. The inhibitor for use according to claims 12 or 13, wherein the subject is suffering from

15 a) acute or chronic inflammation of joints, skin and gut; and/or
b) autoimmune diseases, rheumatoid arthritis, psoriasis, psoriatic arthritis, juvenile arthritis, ankylosing spondylitis, Crohn's disease, multiple sclerosis, congestive heart failure, metabolic disease, cytokine release syndrome, septic shock, acute and chronic neurodegenerative disease, stroke, Alzheimer and Parkinson disease, colitis
20 ulcerosa, pancreatitis, COPD, acute fulminant viral or bacterial infections, metabolic diseases, chronic neurodegenerative diseases, genetically inherited diseases with TNF/TNFR1 as the causative pathologic mediator, periodic fever syndrome, Cherubism, and cancer.

25 15. Use of an inhibitor of any of claims 1 to 9 in the manufacture of a medicament for treating a human subject in need of an anti-TNF therapy.

16. The use of claim 15, wherein the subject is suffering from a disease where anti-TNF therapies or non-biologic DMARD therapeutics are indicated, preferably as
30 first line treatment, or as second line treatment where anti-TNF or non-biologic DMARD therapeutics failed.

17. The use of claim 15 or 16, wherein the subject has developed anti-drug antibodies.

18. The use of any of claims 15 to 17, wherein the subject is suffering from

a) acute or chronic inflammation of joints, skin and gut; and/or

b) autoimmune diseases, rheumatoid arthritis, psoriasis, psoriatic arthritis, juvenile arthritis, ankylosing spondylitis, Crohn's disease, multiple sclerosis, congestive heart failure, metabolic disease, cytokine release syndrome, septic shock, acute and chronic neurodegenerative disease, stroke, Alzheimer and Parkinson disease, colitis ulcerosa, pancreatitis, COPD, acute fulminant viral or bacterial infections, metabolic diseases, chronic neurodegenerative diseases, genetically inherited diseases with TNF/TNFR1 as the causative pathologic mediator, periodic fever syndrome, Cherubism, and cancer.

19. An isolated nucleic acid encoding the inhibitor of any of claims 1 to 9.

20. An expression vector comprising the nucleic acid of claim 19.

21. A recombinant host cell comprising the nucleic acid of claim 19 or the expression vector of claim 20.