

CLAIMS

1. A binding molecule that specifically binds TNFR2, but not TNFR1, where the binding molecule is an Fc γ R independent agonist of TNFR2 and has a valency of at least two for binding TNFR2.
2. The binding molecule of claim 1, wherein the binding molecule has a valency of at least four.
3. The binding molecule of claim 1 or 2, wherein:
 - (a) the binding molecule has a valency of six for TNFR2; or
 - (b) the binding molecule has a valency of four for TNFR2.
4. The binding molecule of any one of the preceding claims, wherein at least one antigen-binding domain specific for TNFR2 of the binding molecule is a single domain binder (sdB) specific for TNFR2, preferably a single domain antibody (sdAb) specific for TNFR2.
5. The binding molecule of claim 4, wherein the at least one sdBr (single domain binder) specific for TNFR2 is a VHH.
6. The binding molecule of any one of the preceding claims, wherein the binding molecule comprises six TNFR2 specific antigen-binding sites that are each VHH antigen-binding domains specific for TNFR2.
7. The binding molecule of claim 6, wherein:
 - (a) the binding molecule comprises six VHH antigen-binding domains and an Fc region, preferably wherein the binding molecule is a hexavalent antibody;
 - (b) the binding molecule comprises two polypeptides, wherein each polypeptide comprises three VHH binding domains specific for TNFR2, preferably wherein the binding molecule is a hexavalent antibody;
 - (c) the binding molecule comprises two polypeptides, wherein each polypeptide comprises three VHH binding domains specific for TNFR2 with each polypeptide further comprising in its C-terminal portion a heavy chain constant region sequence comprising CH2-CH3, but not CH1, preferably wherein the binding molecule is a hexavalent antibody;
or
 - (d) the binding molecule comprises two polypeptides, where each polypeptide comprises three VHH binding domains specific for TNFR2 with each polypeptide further

comprising in its C terminal portion a heavy chain constant region sequence comprising CH2-CH3, but not CH1, and modifications meaning that the Fc domain has reduced effector function, preferably wherein the binding molecule is a hexavalent antibody.

8. The binding molecule of any one of the preceding claims, wherein:
 - (a) the binding molecule comprises an antigen-binding site comprising a VHH domain comprising a set of three CDRs selected from the sets of SEQ ID NOs: 234/235/236; 213/214/215; 216/217/218; 219/220/221; 222/223/224; 225/226/227; 228/229/230; 231/232/233; 234/235/236; 237/238/239; 240/241/242; 243/244/245; 246/247/248; 249/250/251; 252/253/254; 154/155/156; or a variant set of CDRs of any of those sets with up to a total of five amino acid sequence changes, with the variant antigen-binding site retaining the ability to bind TNFR2;
 - (b) the binding molecule comprises an antigen-binding site comprising a set of three CDRs selected from 154/155/156; 160/161/162; 163/164/165; 166/167/168; 169/170/171; 181/182/183; 187/188/189; 193/194/195 or a variant set of any thereof with up to five amino acid sequence changes with the antigen-binding site retaining the ability to bind TNFR2;
 - (c) the binding molecule is hexavalent comprising six VHH antigen-binding sites which are all the same and are an antigen-binding site as defined in any of (a) to (c);
 - (d) the binding molecule is hexavalent comprising two polypeptides, wherein each comprises three VHH antigen-binding sites, wherein all six antigen-binding sites are the same antigen-binding sites which is as defined in any of (a) to (c);
 - (e) the binding molecule is hexavalent comprising six VHH antigen-binding sites, wherein at least two of the VHH antigen-binding sites have a different specificity for TNFR2, with at least one of those specificities conferred by a VHH antigen-binding site as defined in any of (a) to (c);
 - (f) the binding molecule is hexavalent comprising six VHH antigen-binding sites, wherein at least three of the VHH antigen-binding sites have a different specificity for TNFR2, with at least one of those specificities conferred by a VHH antigen-binding site as defined in any of (a) to (c); or
 - (g) the binding molecule is hexavalent comprising six VHH antigen-binding sites, wherein all six VHH antigen-binding sites have a different specificity for TNFR2, with at least one of those specificities conferred by a VHH antigen-binding site as defined in any of (a) to (c).
9. The binding molecule of any one of claims 1 to 8, wherein:

- (a) the binding molecule comprises at least one VHH antigen-binding domain of SEQ ID No: 205; 198 to 204, 206 to 211, and 140 or a variant thereof with at least 90% sequence identity which is still able to bind TNFR2;
- (b) the binding molecule comprises at least one VHH antigen-binding domain selected from those of SEQ ID Nos: 140, 142 to 145, 149, 151, 153, or a variant of any thereof with at least 90% sequence identity which is still able to bind TNFR2;
- (c) the binding molecule comprises two polypeptides which each comprise at least one VHH antigen-binding domain as defined in (a) or (b);
- (d) the binding molecule comprises two polypeptides where each comprise three VHH antigen-binding domains as defined in (a) or (b);
- (e) the binding molecule comprises two polypeptides where each comprise three VHH antigen-binding domains as defined in (a) or (b), wherein all of the antigen-binding domains of the binding molecule specific for TNFR2 are the same; or
- (f) the binding molecule comprises two polypeptides where each comprise three VHH antigen-binding domains as defined in (a) or (b), wherein not all of the VHH antigen-binding domains of the binding molecule specific for TNFR2 are the same.

10. The binding molecule of any one of claims 1 to 7, wherein:

- (a) the binding molecule comprises two polypeptides which each have:
 - (i) the amino acid sequence of SEQ ID NO: 197, except that the VHH domain corresponding to SEQ ID NO: 140 within that sequence is instead the sequence of one of SEQ ID NOs: 205, 198 to 204, and 206 to 211; or
 - (ii) a variant amino acid sequence with at least 90% sequence identity to (i) and which is still able to bind TNFR2;
- (b) the binding molecule comprises two polypeptides which each have the amino acid sequence of SEQ ID NO: 197 or a variant amino acid sequence with at least 90% sequence identity which is still able to bind TNFR2, preferably where each polypeptide comprises three such VHH binding domains;
- (c) the binding molecule comprises two polypeptides which each have:
 - (i) the amino acid sequence of SEQ ID NO: 197, except that the VHH domain corresponding to SEQ ID NO: 140 within that sequence is instead the sequence of one of SEQ ID NOs: 142 to 145, 149, 151, or 153;

- (ii) an amino acid sequence with at least 90% sequence identity to (i) and which is still able to bind TNFR2;
 - (d) any one of (a) to (c) wherein the variant amino acid sequence has from one to five amino acid sequence changes compared to the specific sequence and the binding molecule retains the ability to bind TNFR2, preferably wherein the variant has only one, two or three, and more preferably one amino acid sequence change compared to the specific sequence; or
 - (e) any one of (a) to (c) wherein the variant amino acid sequence has the sequence of amino acids 302 to 405 of SEQ ID NO: 197 replaced with the amino acid sequence of SEQ ID NO: 327.
11. The binding molecule of any one of claims 1 to 7, wherein:
- (a) the binding molecule comprises a polypeptide having the amino acid sequence of SEQ ID NO: 330 or a variant thereof with at least 90% sequence identity that is still able to bind TNFR2;
 - (b) the binding molecule comprises a polypeptide having the amino acid sequence of SEQ ID NO: 331 or a variant thereof with at least 90% sequence identity that is still able to bind TNFR2; or
 - (c) the binding molecule comprises three polypeptides of (a) or three polypeptides of (b).
12. The binding molecule of any one of claims 1 to 3, wherein the binding molecule is an antibody with a valency of four with both “N-terminal” and “C-terminal” orientation binding sites for TNFR2.
13. The binding molecule of claims 1 to 3 or 12, wherein:
- (a) the binding molecule comprises an antigen-binding site comprising a set of six CDRs wherein:
 - (i) the light chain CDRs are selected from a set of CDR1/CDR2/CDR3 selected from SEQ ID NOs: 266/267/268; 269/270/271; 272/273/274; 275/276/277 and the heavy chain CDRs are selected from a set of CDR1/CDR2/CDR3 selected from 282/283/284; 285/286/287; 288/289/290; or 291/292/293; or
 - (ii) the set of six CDRs is a variant of a set of six CDRs of (i) with no more than a total of five amino acid changes compared to the specific six CDRs and wherein the antigen-binding site retains the ability to bind TNFR2;
 - (b) the binding molecule comprises an antigen-binding site comprising a set of six CDRs, wherein:

- (i) the light chain CDR1/CDR2/CDR3 are SEQ ID NOs 69/70/71 and the heavy chain CDR1/CDR2/CDR3 are SEQ ID NOs: 102/103/104;
 - (ii) the set of six CDRs is a variant of a set of six CDRs of (i) with no more than a total of five amino acid changes compared to the specific six CDRs and wherein the antigen-binding site retains the ability to bind TNFR2;
 - (c) the binding molecule comprises an antigen-binding site comprising a set of three light chain CDRs and/or a set of three heavy chain CDRs from those set out in Table 5;
 - (d) the binding molecule comprises a set of six CDRs from a set of six set out in Table 5 or a variant set of six CDRs with no more than five amino acid sequence changes and the variant retaining the ability to bind TNFR2;
 - (e) the binding molecule comprises four antigen-binding sites as set out in any one of (a) to (d) with all four antigen-binding sites being the same;
 - (f) the binding molecule comprises four antigen-binding sites as set out in any one of (a) to (d) with all four antigen-binding sites being the same, with the antibody being tetravalent for TNFR2; or
 - (g) the binding molecule comprises four antigen-binding sites as set out in any one of (a) to (d) with all four antigen-binding sites being the same, with the antibody being tetravalent for TNFR2, with two N-terminal orientated antigen-binding sites and two C-terminal orientated antigen-binding sites.
14. The binding molecule of claims 1 to 3 or 12, wherein the binding molecule is an antibody comprising:
- (a) two light chain polypeptides which are:
 - (i) two light chain polypeptides wherein each light chain polypeptide comprises an amino acid sequence having the sequence of SEQ ID NO: 261 except that the amino acid sequence corresponding to SEQ ID NO: 295 is replaced with a sequence selected from one of SEQ ID NOs: 262 to 265; or
 - (ii) two variant light chain polypeptides which each have at least 90% sequence identity to (i); and
 - (b) two heavy chain polypeptides which are:
 - (iii) two heavy chain polypeptides wherein each heavy chain polypeptide comprises an amino acid sequence having the sequence of SEQ ID NO: 259 except that the amino acid sequence corresponding to SEQ ID NO: 296 is replaced with a sequence selected from one of SEQ ID NOs: 278 to 281; or
 - (iv) two variant heavy chain polypeptides which each have at least 90% sequence identity to (iii).

15. The binding molecule of any one of claims 1 to 3 or 12, wherein the binding molecule is an antibody comprising:
- (a) two light chain polypeptides having the sequence of SEQ ID NO: 261 or an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO: 261; or
 - (b) two heavy chain polypeptides having the sequence of SEQ ID NO: 259 or an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO: 259.
16. The binding molecule of one of claims 1 to 3 or 12, wherein the binding molecule:
- (a) comprises a light chain of SEQ ID NO: 332 and heavy chain of SEQ ID NO: 333 or variants thereof with at least 90% sequence identity where the binding molecule is still able to act as a TNFR2 agonist;
 - (b) comprises a light chain of SEQ ID NO: 334 and heavy chain of SEQ ID NO: 335 or variants thereof with at least 90% sequence identity where the binding molecule is still able to act as a TNFR2 agonist;
 - (c) comprises a light chain of SEQ ID NO: 336 and heavy chain of SEQ ID NO: 337 or variants thereof with at least 90% sequence identity where the binding molecule is still able to act as a TNFR2 agonist; or
 - (d) comprises two light and two heavy chains all as defined in (a), (b), or (c).
17. The binding molecule of any one of the preceding claims which is able to:
- (a) oligomerise TNFR2 on the surface of a cell expressing TNFR2, preferably to cluster six or more TNFR2 molecules on the cell surface;
 - (b) trigger TNFR2 signalling; and/or
 - (c) stimulate proliferation of leucocytes, preferably T cells, more preferably Treg cells.
18. The binding molecule of claim 1, wherein:
- (a) the binding molecule comprises a moiety that alters serum half-life, preferably wherein the moiety is selected from the group consisting of a Fc tail, serum albumin, a moiety which is a binder of serum albumin, and PEG;
 - (a) the binding molecule is bivalent for TNFR2 binding;
 - (b) the binding molecule is trivalent for TNFR2 binding;
 - (c) the binding molecule is tetravalent for TNFR2 binding; or
 - (d) the binding molecule which has a valency of at least four for TNFR2 binding.

19. The binding molecule of claim 1, wherein:
 - (a) the binding molecule is an antibody;
 - (b) the binding molecule is an antibody which comprises a ligand for TNFR2;
 - (c) the binding molecule comprises at least part of an antibody constant region, preferably an Fc region; or
 - (d) the binding molecule comprises a ligand for TNFR2, preferably wherein the ligand is derived from TNF α or lymphotoxin-alpha, preferably where the binding molecule is:
 - (a) an antibody that comprises a TNFR2 binding site at both the N-terminus and C-terminus of one or more heavy chain of the antibody; or
 - (b) an antibody that comprises a TNFR2 binding site at both the N-terminus and C-terminus of one or more light chain of the antibody.
20. The binding molecule of claim 19, wherein:
 - (a) the antibody is a bivalent antibody;
 - (b) the antibody is a trivalent antibody;
 - (c) the antibody is tetravalent antibody;
 - (d) the antibody is a hexavalent antibody;
 - (e) the antibody is a heavy chain only antibody;
 - (f) the antibody is a single domain antibody, such as a single domain Ab (sdAb), VHH, or a Nanobody;
 - (g) the antibody is a multimer of at least two antibodies;
 - (h) the antibody comprises, or is linked to, a multimerizing moiety preferably tenascin-c peptide (TNC), preferably where the antibody is a multimer of at least two antibodies associated through the tenascin-c peptide sequence;
 - (i) the antibody is linked to one or more molecules derived from a ligand of TNFR2, preferably derived from TNF- α or lymphotoxin alpha.
21. The binding molecule of claim 19 or 20 wherein:
 - (a) the antibody lacks an Fc region;
 - (b) the antibody comprises a modified Fc region that is unable to bind Fc receptors;
or
 - (c) the antibody comprises heavy chain constant regions lacking CH1.
22. The binding molecule of claim 1, wherein the binding molecule is an antibody in an antibody format selected from:
 - IgG with an Fc modification eliminating Fc receptor binding;

- scFv:IgG where the IgG has an Fc modification eliminating Fc receptor binding;
 - IgG-HC:TNC, where the IgG has a modification eliminating Fc receptor binding;
 - scFv:IgG-HC:TNC where the IgG has a modification eliminating Fc receptor binding;
 - hexameric IgG;
 - 3(VHH-TNC);
- wherein:
- HC represents a heavy chain of an IgG;
 - LC represents a light chain of an IgG;
 - scFv:IgG represents a scFv either linked to, or forming part of an IgG molecule;
 - HC:TNC indicates a heavy chain (HC) comprising, or linked to, tenascin-c trimerization domain peptide (TNC);
 - VHH-TNC indicates tenascin linked or bound to a VHH.

23. The binding molecule of claim 1, wherein the binding molecule is an antibody in an antibody format selected from the group consisting of:

- Fab-LC:scFv;
- IgG-HC:scFv where the IgG has a modification eliminating Fc receptor binding, preferably N297A;
- IgG-LC:scFv where the IgG has a modification eliminating Fc receptor binding, preferably N297A;
- IgG-LC:scFv-HC:scFv;
- LC:scFv-HC:scFv-IgG-HC:scFv;
- LC:scFv-HC:scFv-IgG-LC:scFv;
- LC:scFv-HC:scFv-IgG-LC:scFv-HC:scFv; and
- IgG-HC-TNC-scFv;
- sdAb/VHH;
- 3(VHH-TNC),

wherein:

- HC represents a heavy chain of an IgG;
- LC represents a light chain of an IgG;
- LC:scFv indicates a scFv linked to, or forming part of, a light chain;
- HC:scFv indicates a scFv linked to, or forming part of, a heavy chain;
- HC:TNC indicates a heavy chain (HC) comprising, or linked to, tenascin (TNC);
- TNC-scFv indicating tenascin linked or bound to a scFv;
- TNC-VHH indicating tenascin linked or bound to a VHH;
- sdAb indicates a single domain antibody;

- VHH indicates the variable region of a camelid antibody;
- VHH-TNC indicates tenascin linked or bound to a VHH, preferably wherein the binding molecule is an antibody and is in an antibody format selected from:
 - a Fab-scFv;
 - IgG-HC:scFv;
 - LC:scFv-HC:scFv-IgG1; and
 - LC:scFv-HC:scFv-IgG1-TNC.

24. The binding molecule of claim 1 wherein:

- (a) the binding molecule comprises at least two antigen-binding sites with a different specificity for TNFR2;
- (b) all of the antigen-binding sites of the binding molecule that are specific for TNFR2 each have a different specificity for TNFR2;
- (c) the binding molecule comprises two polypeptides, wherein each polypeptide comprises three VHH antigen-binding domains specific for TNFR2 wherein at least two of those three VHH antigen-binding domains have a different specificity for TNFR2; or
- (d) the binding molecule comprises two polypeptides, wherein each polypeptide comprises three VHH antigen-binding domains specific for TNFR2 wherein the three VHH antigen-binding domains have a different specificity for TNFR2.

25. A binding molecule that specifically binds TNFR2, but not TNFR1, where the binding molecule is an FcγR independent agonist of TNFR2, has a valency of at least two for binding TNFR2, and comprises polypeptides comprising mutant TNF-alpha that bind TNFR2, but not TNFR1, and an Fc region, or a tenascin peptide sequence, or both, that oligomerises the polypeptides,

preferably wherein the binding molecule is one:

- (a) which does not comprise antibody sequences;
- (b) which comprises polypeptides comprising mutant TNF-alpha that binds TNFR2, but not TNFR1, and a tenascin peptide;
- (c) wherein the TNF-alpha mutants are TNF80; and/or
- (d) has a valency for TNFR2 of at least three, preferably having a valency for TNFR2 of six

26. The binding molecule of any one of the preceding claims for use in the treatment of the human or animal body.

27. Use of a binding molecule of any one of claims 1 to 25 in the manufacture of a medicament for treating or preventing an autoimmune disorder, or an inflammatory disorder, preferably wherein:
- (a) the disorder is graft versus host disease (GvHD), preferably where the binding molecule is for use in a method where it is administered prior to, at the same time, or after a transplant of a cell, tissue, or organ; or
 - (b) the disorder is one involving dysfunction or unwanted proliferation of leukocytes, preferably of T cells, more preferably of Treg cells; or
 - (c) the disorder is selected from Inflammatory Bowel Disease (such as Ulcerative Colitis, Crohn's Disease, or Celiac Disease), atherosclerosis, lupus, multiple sclerosis, Type 1 Diabetes, myasthenia gravis, pemphigus vulgaris, and bullous pemphigoid.
28. Use of a binding molecule according to any one of claims 1 to 25 in the manufacture of a medicament for stimulating cell proliferation comprising contacting a target cell expressing TNFR2 with a binding molecule according to any one of claims 1 to 25.
29. An *in vitro* or *ex vivo* method of stimulating cell proliferation comprising contacting a target cell expressing TNFR2 with a binding molecule according to any one of claims 1 to 25.
30. A pharmaceutical composition comprising a binding molecule according to any one of claims 1 to 25 and a pharmaceutically acceptable carrier.
31. A method of detecting TNFR2 comprising contacting a test sample with a binding molecule according to any one of claims 1 to 25 and detecting binding of the binding molecule to TNFR2,
- preferably wherein the binding molecule is labelled and the binding of the binding molecule to TNFR2 is detected via the label.
32. Use of a binding molecule according to any one of claims 1 to 25 for the manufacture of a medicament for diagnosis, the method comprising administering the binding molecule to a subject and detecting binding of the binding molecule to TNFR2,
- preferably wherein the binding molecule is labelled and the binding of the binding molecule to TNFR2 is detected via the label.