

More specifically, the disclosure relates to antibodies that bind to human TNFR2, and to their use to modulate the TNF-TNFR2 axis for cancer immunotherapy. The disclosed antibodies may be particularly beneficial for tumor microenvironments enriched in exhausted T cells, suppressive myeloid cells, or regulatory T cells that contribute to anti-PD-1/PD-L1 resistance.

[0012a] In one aspect, the present disclosure provides an anti-TNFR2 antibody comprising: (a) VH: CDR1: SEQ ID NO: 31, CDR2: SEQ ID NO: 32, CDR3: SEQ ID NO: 33; and VL: CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 35, CDR3: SEQ ID NO: 36; (b) VH: CDR1: SEQ ID NO: 19, CDR2: SEQ ID NO: 20, CDR3: SEQ ID NO: 21; and VL: CDR1: SEQ ID NO: 22, CDR2: SEQ ID NO: 23, CDR3: SEQ ID NO: 24; (c) VH: CDR1: SEQ ID NO: 25, CDR2: SEQ ID NO: 26, CDR3: SEQ ID NO: 27; and VL: CDR1: SEQ ID NO: 28, CDR2: SEQ ID NO: 29, CDR3: SEQ ID NO: 30; (d) VH: CDR1: SEQ ID NO: 13, CDR2: SEQ ID NO: 14, CDR3: SEQ ID NO: 15; and VL: CDR1: SEQ ID NO: 16, CDR2: SEQ ID NO: 17, CDR3: SEQ ID NO: 18; (e) VH: CDR1: SEQ ID NO: 37, CDR2: SEQ ID NO: 38, CDR3: SEQ ID NO: 39; and VL: CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 40, CDR3: SEQ ID NO: 41; (f) VH: CDR1: SEQ ID NO: 37, CDR2: SEQ ID NO: 49, CDR3: SEQ ID NO: 39; and VL: CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 40, CDR3: SEQ ID NO: 41; or (g) VH: CDR1: SEQ ID NO: 42, CDR2: SEQ ID NO: 43, CDR3: SEQ ID NO: 44; and VL: CDR1: SEQ ID NO: 45, CDR2: SEQ ID NO: 46, CDR3: SEQ ID NO: 47.

[0013] According to some [embodimentexamples](#), the antibody or antibody fragments comprise a set of six complementarity determining region (CDR) sequences selected from the group consisting of three CDRs of a heavy chain (HC) variable region selected from SEQ ID NOs: 1, 3, 5, 7, 9, 11 and 48, and three light CDRs of a light chain (LC) variable region selected from SEQ ID NOs: 2, 4, 6, 8, 10 and 12, or an analog or derivative thereof having at least 90% sequence identity with the identified antibody or fragment sequence.

[0014] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a heavy chain variable region comprising CDR1: SEQ ID NO: 13, CDR2: SEQ ID NO: 14 and CDR3: SEQ ID NO: 15; and/or a light chain variable region comprising CDR1: SEQ ID NO: 16, CDR2: SEQ ID NO: 17, and CDR3: SEQ ID NO: 18.

[0015] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a heavy chain variable region comprising CDR1: SEQ ID NO: 19, CDR2: SEQ ID NO: 20, and CDR3: SEQ ID NO: 21; and/or a light chain variable region comprising CDR1: SEQ ID NO: 22, CDR2: SEQ ID NO: 23, and CDR3: SEQ ID NO: 24.

[0016] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a heavy chain variable region comprising CDR1: SEQ ID NO: 25, CDR2: SEQ ID NO: 26, and CDR3: SEQ ID NO: 27; and/or a light chain variable region comprising CDR1: SEQ ID NO: 28, CDR2: SEQ ID NO: 29, and CDR3: SEQ ID NO: 30.

[0017] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a heavy chain variable region comprising CDR1: SEQ ID NO: 31, CDR2: SEQ ID NO: 32, and CDR3: SEQ ID NO: 33; and/or a light chain variable region comprising CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 35, and CDR3: SEQ ID NO: 36.

[0018] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a heavy chain variable region comprising CDR1: SEQ ID NO:37, CDR2: SEQ ID NO: 38, and CDR3: SEQ ID NO: 39; and/or a light chain variable region comprising CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 40, and CDR3: SEQ ID NO: 41.

[0019] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a heavy chain variable region comprising CDR1: SEQ ID NO:37, CDR2: SEQ ID NO: 49, and CDR3: SEQ ID NO: 39; and/or a light chain variable region comprising CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 40, and CDR3: SEQ ID NO: 41.

[0020] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a heavy chain variable region comprising CDR1: SEQ ID NO: 42, CDR2: SEQ ID NO: 43, and CDR3: SEQ ID NO: 44; and/or a light chain variable region comprising CDR1: SEQ ID NO: 45, CDR2: SEQ ID NO: 46, and CDR3: SEQ ID NO: 47.

[0021] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a variable heavy chain sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9,11, and 48.

[0022] In other [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a variable light chain sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, and 12.

[0023] In other [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof comprise a variable heavy chain sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11 and 48 and a variable light chain sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10 and 12.

[0024] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragment comprises a variable heavy chain sequence and a variable light chain sequence, selected from the following combinations:

[0025] (a) a variable heavy chain sequence comprising SEQ ID NO: 1 and a variable light chain sequence comprising SEQ ID NO: 2;

[0026] (b) a variable heavy chain sequence comprising SEQ ID NO: 3 and a variable light chain sequence comprising SEQ ID NO: 4;

[0027] (c) a variable heavy chain sequence comprising SEQ ID NO: 5 and a variable light chain sequence comprising SEQ ID NO: 6;

[0028] (d) a variable heavy chain sequence comprising SEQ ID NO: 7 and a variable light chain sequence comprising SEQ ID NO: 8;

[0029] (e) a variable heavy chain sequence comprising SEQ ID NO: 9 and a variable light chain sequence comprising SEQ ID NO: 10;

[0030] (f) a variable heavy chain sequence comprising SEQ ID NO: 48 and a variable light chain sequence comprising SEQ ID NO: 10; and

[0031] (g) a variable heavy chain sequence comprising SEQ ID NO: 11 and a variable light chain sequence comprising SEQ ID NO: 12.

[0032] In some [embodiment examples](#), an anti-TNFR2 antibody is provided, wherein the antibody comprises (a) a heavy chain variable region comprising CDR1: SEQ ID NO: 13, CDR2: SEQ ID NO: 14, and CDR3: SEQ ID NO: 15; and/or a light chain variable region comprising CDR1: SEQ ID NO: 16, CDR2: SEQ ID NO: 17, and CDR3: SEQ ID NO: 18; (b) a heavy chain variable region comprising CDR1: SEQ ID NO: 19, CDR2: SEQ ID NO: 20, and CDR3: SEQ ID NO: 21; and/or a light chain variable region comprising CDR1: SEQ ID NO: 22, CDR2: SEQ ID NO: 23, and CDR3: SEQ ID NO: 24; (c) a heavy chain variable region comprising CDR1: SEQ ID NO: 25, CDR2: SEQ ID NO: 26, and CDR3: SEQ ID NO: 27; and/or a light chain variable region comprising CDR1: SEQ ID NO: 28, CDR2: SEQ ID NO: 29, and CDR3: SEQ ID NO: 30; (d) a heavy chain variable region comprising CDR1: SEQ ID NO: 31, CDR2: SEQ ID NO: 32, and CDR3: SEQ ID NO: 33; and/or a light chain variable region comprising CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 35, and CDR3: SEQ ID NO: 36; (e) a heavy chain variable region comprising CDR1: SEQ ID NO: 37, CDR2: SEQ ID NO: 38, and CDR3: SEQ ID NO: 39; and/or a light chain variable region comprising CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 40, and CDR3: SEQ ID NO: 41; (f) a heavy chain variable region comprising CDR1: SEQ ID NO: 37,

CDR2: SEQ ID NO: 49, and CDR3: SEQ ID NO: 39; and/or a light chain variable region comprising CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 40, and CDR3: SEQ ID NO: 41; or (g) a heavy chain variable region comprising CDR1: SEQ ID NO: 42, CDR2: SEQ ID NO: 43, and CDR3: SEQ ID NO: 44; and/or a light chain variable region comprising CDR1: SEQ ID NO: 45, CDR2: SEQ ID NO: 46, and CDR3: SEQ ID NO: 47.

[0033] In some [embodimentexamples](#), the anti-TNFR2 antibodies and antibody fragments thereof comprise one or more heavy chain variable region CDRs disclosed in Table 1 and/or one or more light chain variable region CDRs disclosed in Table 2.

[0034] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments thereof exhibit one or more of the following structural and functional characteristics, alone or in combination: (a) is specific for human TNFR2 (b) does not bind to human TNFR1, (c) binds to an epitope in the CRD3 or CRD4 region of the N-terminal cysteine-rich domain of TNFR2, (d) cross-reacts with cynomolgus TNFR2 (e) disrupts the human TNF binding interaction, (f) inhibits the soluble TNF α -stimulated T cell activation in the absence of binding to an Fc receptor, (g) inhibits the transmembrane TNF-stimulated T cell activation in the absence of binding to an Fc receptor, (h) enhances agonistic activity in chronically stimulated human effector T cells when binding to an Fc receptor, (i) demonstrates anti-tumor efficacy in a human TNFR2 knock-in MC38 syngeneic tumor model, (j) enhances the tumor growth inhibition of anti-PD-L1 treatment in a human TNFR2 knock-in MC38 tumor model, (k) enhances the efficacy of anti-PD-L1 treatment in a human TNFR2 Knock-in PD1 resistant B16F10 melanoma model, or demonstrates ADCC activity to contribute to anti-tumor activity, or (m) enhances the CD8 to Treg ratio within tumors.

[0035] In some [embodimentexamples](#), the anti-TNFR2 antibodies specifically bind to human cells expressing endogenous levels of TNFR2 and to host cells engineered to overexpress TNFR2, and do not demonstrate binding to cells expressing human TNFR1. The anti-TNFR2 antibodies or antibody fragments disclosed herein bind to cells overexpressing human or cyno TNFR2 with subnanomolar EC₅₀ values.

[0036] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments bind to an epitope in the CRD3 or CRD4 region of the N-terminal cysteine-rich domain of TNFR2.

In alternative [embodimentexamples](#), the anti-TNFR2 antibodies and antibody fragments thereof bind to an epitope in the CRD1 or CRD2 region.

[0037] In some [embodimentexamples](#), the anti-TNFR2 antibodies or antibody fragments cross-react with cynomolgus monkey TNFR2 (cynoTNFR2).

[0038] In some [embodimentexamples](#), the anti-TNFR2 antibodies and antibody fragments thereof block the human TNF/TNFR2 binding interaction. In alternative [embodimentexamples](#), the anti-TNFR2 antibodies and antibody fragments thereof do not block the human TNF/TNFR2 binding interaction but antagonize the activity of soluble TNF and the membrane TNF.

[0039] In some [embodimentexamples](#), the anti-TNFR2 antibodies and antibody fragments thereof inhibit both the soluble TNF α - and the membrane TNF α -stimulated response of human cells expressing TNFR2.

[0040] In some [embodimentexamples](#), the anti-TNFR2 antibodies and antibody fragments thereof comprise a Fc region that is engineered to increase multivalent cross-linking activity with Fc γ Rs, which will enhance the Fc-dependent agonist activity of T cells.

[0041] In some [embodimentexamples](#), the anti-TNFR2 antibodies enhance cytokine secretion by exhausted human effector T cells.

[0042] In some [embodimentexamples](#), the anti-TNFR2 antibodies demonstrate anti-tumor efficacy in a human TNFR2 knock-in MC38 syngeneic murine tumor model.

[0043] In some [embodimentexamples](#), the anti-TNFR2 antibodies enhance the tumor growth inhibition of anti-PD-L1 treatment in a human TNFR2 knock-in MC38 tumor model.

[0044] In some [embodimentexamples](#), the anti-TNFR2 antibodies enhance the efficacy of anti-PD-L1 treatment in a human TNFR2 Knock-in PD1 resistant B16F10 melanoma model.

[0045] In some [embodimentexamples](#), the anti-tumor efficacy of the disclosed anti-TNFR2 antibodies can be achieved by ADCC-mediated depletion of T regulatory cells from the tumor microenvironment.

[0046] In some [embodiment examples](#), the anti-tumor efficacy of the disclosed anti-TNFR2 antibodies can be achieved by enhancing the CD8 to Treg ratio in the tumor microenvironment.

[0047] The present disclosure also provides isolated nucleotide sequences encoding at least one of the above antibody molecules.

[0048] The present disclosure also provides plasmids comprising at least one of the above nucleotide sequences.

[0049] The present disclosure also provides cells comprising one of the above nucleotide sequences, or one of the above plasmids.

[0050] The present disclosure also provides pharmaceutical compositions comprising or consisting of at least one of the antibodies or fragments thereof disclosed herein, and optionally a pharmaceutically acceptable diluent, carrier, vehicle and/or excipient. Such a pharmaceutical composition may be used for the antibody-based immunotherapy of cancer.

[\[0050a\] In one aspect, the present disclosure provides a pharmaceutical composition comprising as an active ingredient, at least one antibody as disclosed herein and a pharmaceutically acceptable carrier.](#)

[\[0050b\] In one aspect, the present disclosure provides an isolated polynucleotide comprising a sequence encoding an anti-TNFR2 antibody as disclosed herein.](#)

[\[0050c\] In one aspect, the present disclosure provides a vector comprising a polynucleotide as disclosed herein.](#)

[\[0050d\] In one aspect, the present disclosure provides a host cell comprising a polynucleotide as disclosed herein, and/or a vector as disclosed herein.](#)

[\[0050e\] In one aspect, the present disclosure provides a method for the production of an anti-TNFR2 antibody as disclosed herein, the method comprising culturing the host cell as disclosed herein.](#)

[0050f] In one aspect, the present disclosure provides the use of a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer.

[0050g] In one aspect, the present disclosure provides the pharmaceutical composition as disclosed herein for use in therapy.

[0051] The present disclosure also relates to methods for treatment of cancer in a patient comprising administering to the patient a therapeutically effective amount of at least one of the disclosed anti-TNFR2 antibodies or fragments thereof, alone or in combination with another therapeutic agent.

BRIEF DESCRIPTION OF THE OF THE DRAWINGS

[0052] The foregoing summary, as well as the following detailed description of the disclosure, will be better understood when read in conjunction with the appended figures. For the purpose of illustrating the disclosure, shown in the figures are embodiments which are presently preferred. It should be understood, however, that the disclosure is not limited to the precise arrangements, examples and instrumentalities shown.

[0053] Figure 1 provides the amino acid sequences of the VH and VL domains of the human anti-TNFR2 antibodies and their respective CDR sequences (Kabat numbering). Sequence identifiers are provided and the CDRs are underlined in the variable domain sequences.

WHAT IS CLAIMED IS:

1. An anti-TNFR2 antibody comprising:

(a) VH: CDR1: SEQ ID NO: ~~3143~~, CDR2: SEQ ID NO: ~~1432~~, CDR3: SEQ ID NO: ~~1533~~; and VL: CDR1: SEQ ID NO: ~~3446~~, CDR2: SEQ ID NO: ~~1735~~, CDR3: SEQ ID NO: ~~1836~~;

(b) VH: CDR1: SEQ ID NO: 19, CDR2: SEQ ID NO: 20, CDR3: SEQ ID NO: 21; and, VL: CDR1: SEQ ID NO: 22, CDR2: SEQ ID NO: 23, CDR3: SEQ ID NO: 24;

(c) VH: CDR1: SEQ ID NO: 25, CDR2: SEQ ID NO: 26, CDR3: SEQ ID NO: 27; and, VL: CDR1: SEQ ID NO: 28, CDR2: SEQ ID NO: 29, CDR3: SEQ ID NO: 30;

(d) VH: CDR1: SEQ ID NO: ~~1334~~, CDR2: SEQ ID NO: ~~1432~~, CDR3: SEQ ID NO: ~~3315~~; and VL: CDR1: SEQ ID NO: ~~1634~~, CDR2: SEQ ID NO: ~~1735~~, CDR3: SEQ ID NO: ~~1836~~;

(e) VH: CDR1: SEQ ID NO: 37, CDR2: SEQ ID NO: 38, CDR3: SEQ ID NO: 39; and, VL: CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 40, CDR3: SEQ ID NO: 41;

(f) VH: CDR1: SEQ ID NO: 37, CDR2: SEQ ID NO: 49, CDR3: SEQ ID NO: 39; and -VL: CDR1: SEQ ID NO: 34, CDR2: SEQ ID NO: 40, CDR3: SEQ ID NO: 41; or

(g) VH: CDR1: SEQ ID NO: 42, CDR2: SEQ ID NO: 43, CDR3: SEQ ID NO: 44; and, VL: CDR1: SEQ ID NO: 45, CDR2: SEQ ID NO: 46, CDR3: SEQ ID NO: 47.

2. The anti-TNFR2 antibody of claim 1, wherein the antibody comprises:

(a) a heavy chain variable region having a sequence set forth in SEQ ID NO: ~~74~~ and a light chain variable region having a sequence set forth in SEQ ID NO: ~~28~~;

- (b) a heavy chain variable region having a sequence set forth in SEQ ID NO: 3 and a light chain variable region having a sequence set forth in SEQ ID NO: 4;
 - (c) a heavy chain variable region having a sequence set forth in SEQ ID NO: 5 and a light chain variable region having a sequence set forth in SEQ ID NO: 6;
 - (d) a heavy chain variable region having a sequence set forth in SEQ ID NO: ~~7~~1 and a light chain variable region having a sequence set forth in SEQ ID NO: ~~8~~2;
 - (e) a heavy chain variable region having a sequence set forth in SEQ ID NO: 9 and a light chain variable region having a sequence set forth in SEQ ID NO: 10;
 - (f) a heavy chain variable region having a sequence set forth in SEQ ID NO: 48 and a light chain variable region having a sequence set forth in SEQ ID NO: 10; or
 - (g) a heavy chain variable region having a sequence set forth in SEQ ID NO: 11 and a light chain variable region having a sequence set forth in SEQ ID NO: 12.
3. The anti-TNFR2 antibody of claim 2, wherein the antibody comprises a heavy chain constant region as set forth in SEQ ID NO: 50.
4. The anti-TNFR2 antibody of claim 2, wherein the antibody comprises a light chain constant region as set forth in SEQ ID NO: 51.
5. The anti-TNFR2 antibody of claim 1, wherein the antibody is a fully human antibody.
6. The anti-TNFR2 antibody of claim 1, wherein the antibody is a chimeric antibody.
7. The anti-TNFR2 antibody of claim 1, wherein the antibody is a bispecific antibody or multispecific antibody.
8. The anti-TNFR2 antibody of claim 1, wherein the antibody is a humanized antibody.
9. The anti-TNFR2 antibody of claim 1, wherein the antibody is an antibody fragment.
10. The anti-TNFR2 antibody of claim 9, wherein the antibody fragment is selected from the group consisting of: Fab, Fab', F(ab)2, Fd, Fv, scFv and scFv-Fc fragment, a single-chain antibody, a minibody, and a diabody.

11. A pharmaceutical composition comprising as an active ingredient, at least one antibody according to anyone of claims 1 to 10 and a pharmaceutically acceptable carrier.
12. The pharmaceutical composition according to claim 11 for use in modulating the immune system by inhibiting binding of ~~anti~~-TNFR2 to TNF- α .
13. The pharmaceutical composition according to any one of claims 11 or 12 for use in ~~treating cancer~~therapy.
14. Use of a pharmaceutical composition according to claim 11 or 12 in the manufacture of a medicament for ~~A method of treating cancer comprising administering to a subject in need thereof, a pharmaceutical composition according to claim 11 or 12.~~
15. An isolated polynucleotide comprising a sequence encoding an anti-TNFR2 antibody according to claim 1.
16. An isolated polynucleotide according to claim 15, encoding a sequence as set forth in any one of SEQ ID NOS: 1 to 12.
17. A vector comprising a polynucleotide according to claim 16.
18. A host cell comprising a polynucleotide according to claim 16, and/or a vector according to claim 17.
19. A method for the production of an anti-TNFR2 antibody according to claim 1, the method comprising culturing the host cell of claim 18.