

In one embodiment, the costimulatory TNF receptor family member is OX40. The disclosure, in one aspect, provides for an agonistic bispecific antibody, comprising

(a) two Fab fragments capable of specific binding to a OX40 comprising

(a) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:2, CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, CDR-H3 comprising the amino acid sequence of SEQ ID NO:6 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:13, CDR-H2 comprising the amino acid sequence of SEQ ID NO:16 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:19,

(b) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:2, CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, CDR-H3 comprising the amino acid sequence of SEQ ID NO:7 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:13, CDR-H2 comprising the amino acid sequence of SEQ ID NO:16 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:20,

(c) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:2, CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, CDR-H3 comprising the amino acid sequence of SEQ ID NO:8 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:13, CDR-H2 comprising the amino acid sequence of SEQ ID NO:16 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:21,

(d) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:2, CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, CDR-H3 comprising the amino acid sequence of SEQ ID NO:9 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:13, CDR-H2 comprising the amino acid sequence of SEQ ID NO:16 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:22,

(e) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, CDR-H2 comprising the amino acid sequence of SEQ ID NO:5, CDR-H3 comprising the amino acid sequence of SEQ ID NO:10 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:14, CDR-H2 comprising the amino acid sequence of SEQ ID NO:17 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:23, or

(f) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, CDR-H2 comprising the amino acid sequence of SEQ ID NO:5, CDR-H3 comprising the amino acid sequence of SEQ ID NO:11 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:14, CDR-H2 comprising the amino acid sequence of SEQ ID NO:17 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:23,

(b) a moiety capable of specific binding to Fibroblast Activation Protein (FAP), and

(c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

Thus, in a particular embodiment, the moiety capable of specific binding to a costimulatory TNF receptor family member binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:1.

In a further embodiment, provided is a bispecific antigen binding molecule, comprising at least one moiety capable of specific binding to OX40, wherein said moiety comprises a VH domain comprising

(i) a CDR-H1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3,

(ii) a CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:5, and

(iii) a CDR-H3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12,

and a VL domain comprising

(iv) a CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15,

(v) a CDR-L2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18, and

(vi) a CDR-L3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:24.

In another embodiment, the disclosure provides a bispecific antigen binding molecule, wherein the moiety capable of specific binding to OX40 comprises a heavy chain variable region VH comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO: 27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35 and SEQ ID NO:37 and a light chain variable region VL comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence of SEQ ID NO:26, SEQ ID NO: 28, SEQ ID NO:30, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36 and SEQ ID NO:38

Particularly, a bispecific antigen binding molecule is provided, wherein the moiety capable of specific binding to OX40 comprises

- (i) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:25 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:26,

- (v) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:33 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:34,
- (vi) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:35 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:36, or
- (vii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:37 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:38.

In yet another embodiment, provided is an antibody that specifically binds to 4-1BB, wherein said antibody comprises

- (i) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:58 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:59,
- (ii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:60 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:61,
- (iii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:62 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:63,
- (iv) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:64 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:65, or
- (v) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:66 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:67.

According to another embodiment of the disclosure , there is provided an isolated polynucleotide encoding a bispecific antigen binding molecule as described herein before or an antibody that specifically binds to OX40 as described herein before or an antibody that specifically binds to 4-1BB as described herein before. The disclosure, in another aspect, provides a polynucleotide encoding the agonistic bispecific antibody as described herein. The disclosure further provides a vector, particularly an expression vector, comprising the isolated polynucleotide of the disclosure and a host cell comprising the isolated polynucleotide or the vector of the disclosure. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell.

In another embodiment, provided is a method for producing a bispecific antigen binding molecule as described herein before or an antibody that specifically binds to OX40 as described herein before or an antibody that specifically binds to 4-1BB as described herein before, comprising the steps of (i) culturing the host cell of the disclosure under conditions suitable for expression of the antigen binding molecule, and (ii) recovering the antigen binding molecule. The disclosure also encompasses the bispecific antigen binding molecule or the antibody that specifically binds to OX40 or the antibody that specifically binds to 4-1BB produced by the method of the disclosure.

The disclosure further provides a pharmaceutical composition comprising a bispecific antigen binding molecule as described herein before or an antibody that specifically binds to OX40 as described herein before or an antibody that specifically binds to 4-1BB as described herein before and at least one pharmaceutically acceptable excipient. The disclosure, in another aspect, provides a pharmaceutical composition comprising an agonistic bispecific antibody as described herein and at least one pharmaceutically acceptable excipient.

Also encompassed by the disclosure is the bispecific antigen binding molecule as described herein before or the antibody that specifically binds to OX40 as described herein before or the antibody that specifically binds to 4-1BB as described herein before, or the pharmaceutical composition comprising the bispecific antigen binding molecule or the antibody that specifically binds to OX40 or the antibody that specifically binds to 4-1BB, for use as a medicament. The disclosure, in another aspect, provides the agonistic bispecific antibody as described herein or the pharmaceutical composition as described herein, for use as a medicament.

There is provided a bispecific antigen binding molecule as described herein before or an antibody that specifically binds to OX40 as described herein before or an antibody that specifically binds to 4-1BB as described herein before or the pharmaceutical composition of the disclosure, for use

- (i) in stimulating T cell response,
- (ii) in supporting survival of activated T cells,
- (iii) in the treatment of infections,
- (iv) in the treatment of cancer,
- (v) in delaying progression of cancer, or
- (vi) in prolonging the survival of a patient suffering from cancer.

The disclosure, in another aspect, provides the bispecific antigen binding molecule as described herein or the pharmaceutical composition as described herein, for use

- (i) in stimulating T cell response,
- (ii) in supporting survival of activated T cells,
- (iii) in the treatment of infections,
- (iv) in the treatment of cancer, or
- (v) in delaying progression of cancer.

In a specific embodiment, provided is the bispecific antigen binding molecule as described herein before or the antibody that specifically binds to OX40 as described herein before or the antibody that specifically binds to 4-1BB as described herein before, or the pharmaceutical composition of the disclosure , for use in the treatment of cancer.

The disclosure, in another aspect, provides the agonistic bispecific antibody as described herein or the pharmaceutical composition as described herein, for use in the treatment of cancer.

The disclosure also provides the bispecific antigen binding molecule as described herein before or the antibody that specifically binds to OX40 as described herein before or the antibody that specifically binds to 4-1BB as described herein for use in the treatment of cancer, wherein the bispecific antigen binding molecule is administered in

combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy.

The disclosure, in another aspect, provides the agonistic bispecific antibody as described herein or the pharmaceutical composition as described herein, for use in the treatment of cancer, wherein the bispecific antigen binding molecule is to be administered in combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy.

In a further embodiment, the disclosure provides a method of inhibiting the growth of tumor cells in an individual comprising administering to the individual an effective amount of the bispecific antigen binding molecule as described herein before or the antibody that specifically binds to OX40 as described herein before or the antibody that specifically binds to 4-1BB as described herein before, or the pharmaceutical composition of the disclosure, to inhibit the growth of the tumor cells.

Also provided is the use of the bispecific antigen binding molecule as described herein before or the antibody that specifically binds to OX40 as described herein before or the antibody that specifically binds to 4-1BB as described herein before for the manufacture of a medicament for the treatment of a disease in an individual in need thereof, in particular for the manufacture of a medicament for the treatment of cancer, as well as a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the TNF family ligand trimer-containing antigen binding molecule of the disclosure in a pharmaceutically acceptable form. In a specific embodiment, the disease is cancer. In any of the above, the individual is a mammal, particularly a human.

The disclosure, in another aspect, provides use of the agonistic bispecific antibody as described herein or the pharmaceutical composition as described herein, in the manufacture of a medicament for the inhibition of growth of tumor cells or for the treatment of cancer.

Claims

1. An agonistic bispecific antibody, comprising

(a) two Fab fragments capable of specific binding to a OX40 comprising

(a) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:2, CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, CDR-H3 comprising the amino acid sequence of SEQ ID NO:6 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:13, CDR-H2 comprising the amino acid sequence of SEQ ID NO:16 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:19,

(b) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:2, CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, CDR-H3 comprising the amino acid sequence of SEQ ID NO:7 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:13, CDR-H2 comprising the amino acid sequence of SEQ ID NO:16 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:20,

(c) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:2, CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, CDR-H3 comprising the amino acid sequence of SEQ ID NO:8 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:13, CDR-H2 comprising the amino acid sequence of SEQ ID NO:16 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:21,

(d) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:2, CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, CDR-H3 comprising the amino acid sequence of SEQ ID NO:9 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:13, CDR-H2 comprising the amino acid sequence of SEQ ID NO:16 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:22,

(e) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, CDR-H2 comprising the amino acid sequence of SEQ ID NO:5, CDR-H3 comprising the amino acid sequence of SEQ ID NO:10 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:14, CDR-H2 comprising the amino acid sequence of SEQ ID NO:17 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:23, or

(f) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, CDR-H2 comprising the amino acid sequence of SEQ ID NO:5, CDR-

H3 comprising the amino acid sequence of SEQ ID NO:11 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:14, CDR-H2 comprising the amino acid sequence of SEQ ID NO:17 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:23,

- (b) a moiety capable of specific binding to Fibroblast Activation Protein (FAP), and
- (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

2. The agonistic bispecific antibody of claim 1, wherein the Fab fragments capable of specific binding to OX40 comprise

- (i) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:25 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:26,
- (ii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:27 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:28,
- (iii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:29 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:30,
- (iv) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:31 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:32,
- (v) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:33 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:34, or
- (vi) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:35 and and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:36.

3. The agonistic bispecific antibody of claim 1 or 2, wherein the moiety capable of specific binding to FAP comprises

(a) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:68, CDR-H2 comprising the amino acid sequence of SEQ ID NO:70, CDR-H3 comprising the amino acid sequence of SEQ ID NO:72 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:74, CDR-H2 comprising the amino acid sequence of SEQ ID NO:76 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:78, or

(b) a VH domain comprising CDR-H1 comprising the amino acid sequence of SEQ ID NO:69, CDR-H2 comprising the amino acid sequence of SEQ ID NO:71, CDR-H3 comprising the amino acid sequence of SEQ ID NO:73 and a VL domain comprising CDR-L1 comprising the amino acid sequence of SEQ ID NO:75, CDR-H2 comprising the amino acid sequence of SEQ ID NO:77 and CDR-H3 comprising the amino acid sequence of SEQ ID NO:79.

4. The agonistic bispecific antibody of any one of claims 1 to 3, wherein the moiety capable of specific binding to FAP comprises

(i) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:80 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:81, or

(ii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:82 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:83.

5. The agonistic bispecific antibody of any one of claims 1 to 4, wherein

(i) the Fab fragments capable of specific binding to OX40 comprise a heavy chain variable region VH comprising the amino acid sequence of SEQ ID NO: 27 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 28 and

(ii) the moiety capable of specific binding to FAP comprises a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:82 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:83.

6. The agonistic bispecific antibody of any one of claims 1 to 5, wherein the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain.

7. The agonistic bispecific antibody of any one of claims 1 to 6, wherein the first subunit of the Fc domain comprises knobs and the second subunit of the Fc domain comprises holes according to the knobs into holes method.

8. The agonistic bispecific antibody of any one of claims 1 to 7, wherein the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (numbering according to Kabat EU index) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index).

9. The agonistic bispecific antibody of any one of claims 1 to 8, comprising
(a) two Fab fragments capable of specific binding to OX40,
(b) two moieties capable of specific binding to FAP,
and
(c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

10. The agonistic bispecific antibody of claim 9, wherein the bispecific antigen binding molecule is bivalent both for OX40 and for FAP.

11. The agonistic bispecific antibody of any one of claims 1 to 10, comprising
(a) two light chains and two heavy chains of an antibody comprising two Fab fragments capable of specific binding to OX40 and the Fc domain, and
(b) two additional Fab fragments capable of specific binding to FAP, wherein said additional Fab fragments are each connected via a peptide linker to the C-terminus of the heavy chains of (a).

12. The agonistic bispecific antibody of claim 11, wherein the two additional Fab fragments capable of specific binding to FAP are crossover Fab fragments wherein the variable domains VL and VH are replaced by each other and the VL-CH chains are each connected via a peptide linker to the C-terminus of the heavy chains of (a).

13. The agonistic bispecific antibody of any one of claims 1 to 8, comprising
(a) two Fab fragments capable of specific binding to OX40,
(b) one moiety capable of specific binding to FAP,
and
(c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

14. The agonistic bispecific antibody of claim 17, wherein the bispecific antigen binding molecule is bivalent for OX40 and monovalent for FAP.

15. The agonistic bispecific antibody of claims 1 to 8 or 13 or 14, comprising
(a) two light chains and two heavy chains of an antibody comprising two Fab fragments capable of specific binding to OX40 and the Fc domain, and
(b) a VH and VL domain capable of specific binding to a target cell antigen, wherein the VH domain is connected via a peptide linker to the C-terminus of one of the heavy chains and wherein the VL domain is connected via a peptide linker to the C-terminus of the second heavy chain.

16. A polynucleotide encoding the agonistic bispecific antibody of any one of claims 1 to 15.

17. A pharmaceutical composition comprising an agonistic bispecific antibody of any one of claims 1 to 15 and at least one pharmaceutically acceptable excipient.

18. The agonistic bispecific antibody of any one of claims 1 to 15 or the pharmaceutical composition of claim 17, for use as a medicament.

19. The agonistic bispecific antibody of any one of claims 1 to 15 or the pharmaceutical composition of claim 17, for use
(i) in stimulating T cell response,
(ii) in supporting survival of activated T cells,
(iii) in the treatment of infections,
(iv) in the treatment of cancer, or
(v) in delaying progression of cancer.

20. The agonistic bispecific antibody of any one of claims 1 to 15 or the pharmaceutical composition of claim 17, for use in the treatment of cancer.

21. The agonistic bispecific antibody of any one of claims 1 to 15 or the pharmaceutical composition of claim 17, for use in the treatment of cancer, wherein the bispecific antigen binding molecule is to be administered in combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy.

22. Use of the agonistic bispecific antibody of any one of claims 1 to 15 or the pharmaceutical composition of claim 17, in the manufacture of a medicament for the inhibition of growth of tumor cells or for the treatment of cancer.
