

333, 1030-1034; Teng M.W. et al. (2009), J. Immunol. 183, 1911-1920). However, systemic administration of 4-1BB-specific agonistic antibodies with the functionally active Fc domain also induces expansion of CD8⁺ T-cells associated with liver toxicity (Dubrot J. et al. (2010), Cancer Immunol. Immunother. 59, 1223-1233) that is diminished or significantly ameliorated in the absence of functional Fc-receptors in mice. In human clinical trials (ClinicalTrials.gov, NCT00309023), Fc-competent 4-1BB agonistic antibodies (BMS-663513) administered once every three weeks for 12 weeks induced stabilization of the disease in patients with melanoma, ovarian or renal cell carcinoma. However, the same antibody given in another trial (NCT00612664) caused grade 4 hepatitis leading to termination of the trial (Simeone E. and Ascierto P.A. (2012), J. Immunotoxicology 9, 241-247). Thus, there is a need for new generation agonists that should not only effectively engage 4-1BB on the surface of hematopoietic and endothelial cells but also be capable of achieving that through mechanisms other than binding to Fc-receptors in order to avoid uncontrollable side effects.

The available pre-clinical and clinical data clearly demonstrate that there is a high clinical need for effective agonists of costimulatory TNFR family members such as Ox40 and 4-1BB that are able to induce and enhance effective endogenous immune responses to cancer. However, almost never are the effects limited to a single cell type or acting via a single mechanism and studies designed to elucidate inter- and intracellular signaling mechanisms have revealed increasing levels of complexity. Thus, there is a need of “targeted” agonists that preferably act on a single cell type. The antigen binding molecules of the invention combine a moiety capable of preferred binding to tumor-specific or tumor-associated targets with a moiety capable of agonistic binding to costimulatory TNF receptors. The antigen binding molecules of this invention may be able to trigger TNF receptors not only effectively, but also very selectively at the desired site thereby reducing undesirable side effects.

SUMMARY

The present disclosure relates to bispecific antigen binding molecules combining four moieties capable of specific binding to a costimulatory TNF receptor family member, i.e four antigen binding sites that target costimulatory TNF receptors with at least one moiety capable of specific binding to a target cell antigen, i.e. with at least one antigen binding side targeting a target cell antigen. These bispecific antigen binding molecules are advantageous as they will preferably activate costimulatory TNF receptors at the site where the target cell antigen is expressed, due to their binding capability towards a target cell antigen.

The disclosure provides a bispecific antigen binding molecule, comprising
(a) four moieties capable of specific binding to a costimulatory TNF receptor family member,
(b) at least one moiety capable of specific binding to a target cell antigen, and

(c) a Fc domain composed of a first and a second subunit capable of stable association.

In one aspect, the disclosure provides a bispecific antigen binding molecule, comprising (a) four moieties capable of specific binding to a costimulatory TNF receptor family member, (b) at least one moiety capable of specific binding to a target cell antigen, and (c) a Fc domain
5 composed of a first and a second subunit capable of stable association.

In one embodiment, the disclosure provides a bispecific antigen binding molecule, wherein each two of the four moieties capable of specific binding to a costimulatory TNF receptor family member are fused to each other. Optionally, they are fused to each other via a peptide linker. In particular, provided is a bispecific antigen binding molecule, wherein each two of the four
10 moieties capable of specific binding to a costimulatory TNF receptor family member are fused to each other and the C-terminus to the N-terminus of one of the subunits of the Fc domain, optionally they are connected at the C-terminus to the N-terminus of one of the subunits of the Fc domain via a peptide linker.

In a particular embodiment, the disclosure provides a bispecific antigen binding molecule,
15 wherein the molecule comprises two heavy chains and each of the heavy chains comprises variable domains of two moieties capable of specific binding to a costimulatory TNF receptor family member and a variable domain of a moiety capable of specific binding to a target cell antigen.

In a particular embodiment, the bispecific antigen binding molecule comprises (a) four
20 moieties capable of specific binding to a costimulatory TNF receptor family member, wherein the costimulatory TNF receptor family member is selected from the group consisting of OX40, 4-1BB and GITR, (b) at least one moiety capable of specific binding to a target cell antigen, and (c) a Fc domain composed of a first and a second subunit capable of stable association.

In one embodiment, the costimulatory TNF receptor family member is OX40. Thus, in a
25 particular aspect, 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 four moieties capable of specific binding to OX40, wherein said moiety comprises a VH domain comprising
30 (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

- 5 (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 each of the moieties 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 each of the moieties 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 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 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 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 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 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 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 a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:38.

In another embodiment, the bispecific antigen binding molecule comprises (a) four moieties capable of specific binding to a costimulatory TNF receptor family member, (b) at least one moiety capable of specific binding to a target cell antigen, wherein the target cell antigen is

selected from the group consisting of Fibroblast Activation Protein (FAP), Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), Epidermal Growth Factor Receptor (EGFR), Carcinoembryonic Antigen (CEA), CD19, CD20 and CD33, and (c) a Fc domain composed of a first and a second subunit capable of stable association. More particularly, the target cell antigen is Fibroblast Activation Protein (FAP).

In a particular embodiment, provided is a bispecific antigen binding molecule, wherein the moiety capable of specific binding to FAP comprises a VH domain comprising

(i) a CDR-H1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:39 and SEQ ID NO:40,

(ii) a CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:41 and SEQ ID NO:42, and

(iii) a CDR-H3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:43 and SEQ ID NO:44,

and a VL domain comprising

(iv) a CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:45 and SEQ ID NO:46,

(v) a CDR-L2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:48, and

(vi) a CDR-L3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:49 and SEQ ID NO:50.

In another embodiment, provided is a bispecific antigen binding molecule, wherein the moiety capable of specific binding to FAP comprises a VH domain comprising comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:51 or SEQ ID NO:53 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:52 or SEQ ID NO:54.

In a further embodiment, the disclosure thus provides a bispecific antigen binding molecule, wherein

(i) each of the moieties 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 the amino acid sequence 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 or SEQ ID NO:37 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:26, SEQ ID NO: 28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36 or SEQ ID NO:38 and

(ii) the moiety capable of specific binding to FAP 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 the amino acid sequence of SEQ ID NO:51 or SEQ ID NO:53 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:52 or SEQ ID NO:54.

In another embodiment, the costimulatory TNF receptor family member is 4-1BB. Thus, in a particular aspect, 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:239.

In a particular embodiment, provided is a bispecific antigen binding molecule, comprising four moieties capable of specific binding to 4-1BB, wherein each of said moieties comprises a VH domain comprising

(i) a CDR-H1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:249 and SEQ ID NO:250,

(ii) a CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:251 and SEQ ID NO:252, and

(iii) a CDR-H3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO: 256, and SEQ ID NO:257,

and a VL domain comprising

(iv) a CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:258 and SEQ ID NO:259,

(v) a CDR-L2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:260 and SEQ ID NO:261, and

(vi) a CDR-L3 comprising the amino acid sequence selected from the group consisting of

SEQ ID NO:262, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:265, and SEQ ID NO:266.

In a further embodiment, provided is a bispecific antigen binding molecule, wherein each of the moieties capable of specific binding to 4-1BB 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:267, SEQ ID NO: 269, SEQ ID NO:271, SEQ ID NO:273, and SEQ ID NO:275, 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 selected from the group consisting of SEQ ID NO:268, SEQ ID NO: 270, SEQ ID NO:272, SEQ ID NO:274, and SEQ ID NO:276.

In an additional embodiment, provided is a bispecific antigen binding molecule, wherein each of the moieties capable of specific binding to 4-1BB comprises

- (i) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:267 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:268,
- 5 (ii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:269 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:270,
- (iii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:271 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:272,
- 10 (iv) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:273 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:274, or
- (v) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:275 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:276.
- 15

Thus, in a further embodiment, the disclosure provides a bispecific antigen binding molecule, wherein

- (i) each of the moieties capable of specific binding to 4-1BB 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 the amino acid sequence selected from the group consisting of SEQ ID NO:267, SEQ ID NO: 269, SEQ ID NO:271, SEQ ID NO:273, and SEQ ID NO:275 and a light chain variable region 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:268, SEQ ID NO: 270, SEQ ID NO:272, SEQ ID NO:274, and SEQ ID NO:276,
- 20 and
- (ii) the moiety capable of specific binding to FAP 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 the amino acid sequence of SEQ ID NO:51 or SEQ ID NO:53 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:52 or SEQ ID NO:54.
- 30

In yet another embodiment, the disclosure provides a bispecific antigen binding molecule as defined herein before, wherein the costimulatory TNF receptor family member is GITR. Thus, in a particular aspect, 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:357.

In one embodiment, provided is a bispecific antigen binding molecule comprising four moieties capable of specific binding to GITR, wherein each of said moieties comprises a VH domain comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO:371, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:372 and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:373, and a VL domain comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO:374, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:375 and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:376.

In a further embodiment, provided is a bispecific antigen binding molecule, wherein each of the moieties capable of specific binding to GITR 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 of SEQ ID NO:383, 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:384.

In a particular embodiment, provided is a bispecific antigen binding molecule as defined herein before, wherein each of the moieties capable of specific binding to GITR comprises a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:383 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:384.

In another particular embodiment, the disclosure provides a bispecific antigen binding molecule, wherein

(i) each of the moieties capable of specific binding to GITR 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 the amino acid sequence of SEQ ID NO:383, and a light chain variable region 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:384, and

(ii) the moiety capable of specific binding to FAP 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 the amino acid sequence of SEQ ID NO:51 or SEQ ID NO:53 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:52 or SEQ ID NO:54.

In one embodiment, provided is a bispecific antigen binding molecule as defined herein before, wherein the four moieties capable of specific binding to a costimulatory TNF receptor family member are antibody fragments selected from the group of Fab fragments, cross-Fab fragments, single-chain antibody molecules (e.g. scFv), antigen binding domains comprising

the antibody light chain variable region (VL) and the antibody heavy chain variable region (VH) and single domain antibodies (e.g. aVH).

5 In another embodiment, provided is a bispecific antigen binding molecule as defined herein before, wherein the four moieties capable of specific binding to a costimulatory TNF receptor family member are Fab fragments and each two thereof are connected to each other.

10 In a further embodiment, provided is a bispecific antigen binding molecule as described herein before, wherein a first Fab fragment capable of specific binding to a costimulatory TNF receptor family member is fused at the C-terminus of the CH1 domain to the VH domain of a second Fab fragment capable of specific binding to a costimulatory TNF receptor family member and a third Fab fragment capable of specific binding to a costimulatory TNF receptor family member is fused at the C-terminus of the CH1 domain to the VH domain of a fourth Fab fragment capable of specific binding to a costimulatory TNF receptor family member, optionally via a peptide linker.

15 In another embodiment, the disclosure provides a bispecific antigen binding molecule, wherein in the Fab fragments capable of specific binding to a costimulatory TNF receptor family member in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat EU Index), and in the constant domain CH1 the amino acids at positions 147 and 213 are substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

20 In a further embodiment, the disclosure relates to a bispecific antigen binding molecule as described herein before, wherein the bispecific antigen binding molecule is tetravalent for the costimulatory TNF receptor family member and monovalent for the target cell antigen.

25 In one embodiment, provided is a bispecific antigen binding molecule, wherein the moiety capable of specific binding to a target cell antigen comprises a VH and VL domain and wherein the VH domain is connected via a peptide linker to the C-terminus of the first subunit of the Fc domain and the VL domain is connected via a peptide linker to the C-terminus of the second subunit of the Fc domain.

In another embodiment, the disclosure provides a bispecific antigen binding, wherein the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain. In particular, 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. More
5 particularly, 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).

In another embodiment, provided is a bispecific antigen binding molecule of the disclosure, wherein said antigen binding molecule comprises

5 (i) a first heavy chain comprising an amino acid sequence of SEQ ID NO:213, a second heavy chain comprising an amino acid sequence of SEQ ID NO:214, and a light chain comprising an amino acid sequence of SEQ ID NO.157, or

(ii) a first heavy chain comprising an amino acid sequence of SEQ ID NO:217, a second heavy chain comprising an amino acid sequence of SEQ ID NO:218, and a light chain
10 comprising an amino acid sequence of SEQ ID NO.157.

In addition, the disclosure relates to a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule is tetravalent for the costimulatory TNF receptor family member and bivalent for the target cell antigen.

In one embodiment, provided is a bispecific antigen binding molecule, wherein the the two
15 moieties capable of specific binding to a target cell antigen are Fab fragments or crossover Fab fragments.

In a further embodiment, provided is a bispecific antigen binding molecule, wherein each of the Fab fragments or crossover Fab fragments capable of specific binding to a target cell antigen is fused at the N-terminus of the VH or VL domain via a peptide linker to the C-terminus
20 of one of the subunits of the Fc domain.

In a particular embodiment, provided is a bispecific antigen binding molecule, wherein the two moieties capable of specific binding to a target cell antigen are VH-VL crossover Fab fragments and are each fused at the N-terminus of the VL domain via a peptide linker to the C-terminus of one of the subunits of the Fc domain.

25 More particularly, provided is a bispecific antigen binding molecule, wherein said antigen binding molecule comprises (i) a heavy chain comprising an amino acid sequence of SEQ ID NO:227, a first light chain of SEQ ID NO:226 and a second light chain of SEQ ID NO:228, or (ii) a heavy chain comprising an amino acid sequence of SEQ ID NO:224, a first light chain of SEQ ID NO:226 and a second light chain of SEQ ID NO:225.

30 In yet another embodiment, the disclosure provides a bispecific antigen binding, wherein the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

According to another embodiment of the disclosure, there is provided an isolated polynucleotide encoding a bispecific antigen binding molecule as described herein before.

According to another aspect of the disclosure, there is provided a polynucleotide encoding the bispecific antigen binding molecule of the disclosure. The disclosure further provides a vector,
5 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 aspects the host cell is a eukaryotic cell, particularly a mammalian cell.

There is also provided a method for producing a bispecific antigen binding molecule as described herein before, comprising the steps of (i) culturing the host cell of the disclosure under
10 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, or the antibody that specifically binds to GITR, produced by the method of the disclosure.

According to another aspect, the disclosure further provides a pharmaceutical composition
15 comprising a bispecific antigen binding molecule as described herein before and at least one pharmaceutically acceptable excipient.

According to another aspect, the disclosure provides the bispecific antigen binding molecule as described herein before, or the pharmaceutical composition comprising the
20 bispecific antigen binding molecule, for use as a medicament.

In another aspect, provided is a bispecific antigen binding molecule 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,
- 25 (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.

In another aspect, provided is the bispecific antigen binding molecule as described herein before or the pharmaceutical composition of the disclosure, for use in the treatment of cancer. In another embodiment, the disclosure provides the bispecific antigen binding molecule as described herein before 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. According to another aspect, the present disclosure provides for the bispecific antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, 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.

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 pharmaceutical composition of the disclosure, to inhibit the growth of the tumor cells.

Also provided is the use of the the bispecific antigen binding molecule 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 bispecific antigen binding molecule of the disclosure in a pharmaceutically acceptable form. According to another aspect, the present disclosure provides for use of the bispecific antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure in the manufacture of a medicament for the treatment of cancer. In a specific embodiment, the disease is cancer. In any of the above the individual is a mammal, particularly a human.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows the monomeric form of Fc-linked TNF receptor antigen that was used for the preparation of TNF receptor antibodies. **Figure 1B** shows a dimeric human TNF receptor antigen Fc fusion molecule with a C-terminal Ha tag that was used for the testing of the binding of TNF receptor antibodies in the presence of TNF ligand (ligand blocking property). The schematic setup of the experiment described in Example 2.4 is shown in **Figure 1C**.

Figures 2A-2D show the binding of anti-OX40 antibodies to activated human CD4⁺ and CD8⁺ T cells. OX40 is not expressed on resting human PBMCs (Figures 2A and 2C). After activation of human PBMCs, OX40 is up-regulated on CD4⁺ and CD8⁺ T cells (Figures 2B and 2D). OX40 expression on human CD8⁺ T cells is lower than on CD4⁺ T cells. The depicted clones varied in their binding strength (EC₅₀ values as well as signal strength) to OX40 positive cells. Shown is the binding as median of fluorescence intensity (MFI) of FITC labeled anti-human IgG Fcγ-specific goat IgG F(ab')₂ fragment which is used as secondary detection antibody. MFI was measured by flow cytometry and baseline corrected by subtracting the MFI of the blank control. The x-axis shows the concentration of antibody constructs. All OX40 clones do bind to activated, OX40 expressing human CD4⁺ T cells, and to a lower extent to activated human CD8⁺ T cells.

Figures 3A-3D show the binding of the anti-OX40 antibodies to activated mouse CD4⁺ and CD8⁺ T cells. OX40 was not detected on resting mouse splenocytes (Figures 3A and 3C). After activation OX40 is up-regulated on CD4⁺ and CD8⁺ T cells (Figures 3B and 3D). Mouse splenocytes were isolated by erythrolysis with ACK lysis buffer of mechanically-homogenized spleens obtained from 6-8 weeks old female C57BL/6 mice. Binding of anti-OX40 antibodies to

cell surface proteins was detected with a goat anti-human IgG Fc-specific secondary antibody conjugated to FITC using FACS analysis. MFI was measured by flow cytometry and baseline corrected by subtracting the MFI of the blank control. The x-axis shows the concentration of

Claims

1. A bispecific antigen binding molecule, comprising
 - (a) four moieties capable of specific binding to a costimulatory TNF receptor family member,
 - (b) at least one 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.
2. The bispecific antigen binding molecule of claim 1, wherein each two of the four moieties capable of specific binding to a costimulatory TNF receptor family member are fused to each other, optionally via a peptide linker.
3. The bispecific antigen binding molecule of claims 1 or 2, wherein the costimulatory TNF receptor family member is selected from the group consisting of OX40, 4-1BB and GITR.
4. The bispecific antigen binding molecule of any one of claims 1 to 3, wherein the costimulatory TNF receptor family member is OX40.
5. The bispecific antigen binding molecule of any one of claims 1 to 4, wherein 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.
6. The bispecific antigen binding molecule of any one of claims 1 to 5, comprising four moieties capable of specific binding to OX40, wherein each of said moieties 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.

7. The bispecific antigen binding molecule of any one of claims 1 to 6, wherein each of the moieties 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 selected from the group consisting 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.

8. The bispecific antigen binding molecule of any one of claims 1 to 7, wherein each of the moieties 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 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 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 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 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 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 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 a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:38.

9. The bispecific antigen binding molecule of any one of claims 1 to 8, wherein the moiety capable of specific binding to FAP comprises a VH domain comprising

- (i) a CDR-H1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:39 and SEQ ID NO:40,
- (ii) a CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:41 and SEQ ID NO:42, and
- (iii) a CDR-H3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:43 and SEQ ID NO:44,

and a VL domain comprising

- (iv) a CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:45 and SEQ ID NO:46,
- (v) a CDR-L2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:48, and
- (vi) a CDR-L3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:49 and SEQ ID NO:50.

10. The bispecific antigen binding molecule of any one of claims 1 to 9, wherein

- (i) each of the moieties 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 or SEQ ID NO:37 and a light chain variable region 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:26, SEQ ID NO: 28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36 or SEQ ID NO:38 and
- (ii) the moiety capable of specific binding to FAP 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 the amino acid sequence of SEQ ID NO:51 or SEQ ID NO:53 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:52 or SEQ ID NO:54.

11. The bispecific antigen binding molecule of any one of claims 1 to 3 or 9, wherein the costimulatory TNF receptor family member is 4-1BB.

12. The bispecific antigen binding molecule of any one of claims 1 to 3 or 9 or 11, wherein 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:239.

13. The bispecific antigen binding molecule of any one of claims 1 to 3, 9 or 11 or 12, comprising four moieties capable of specific binding to 4-1BB, wherein each of said moieties comprises a VH domain comprising

- (i) a CDR-H1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:249 and SEQ ID NO:250,
- (ii) a CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:251 and SEQ ID NO:252, and
- (iii) a CDR-H3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO: 256, and SEQ ID NO:257,

and a VL domain comprising

- (iv) a CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:258 and SEQ ID NO:259,
- (v) a CDR-L2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:260 and SEQ ID NO:261, and
- (vi) a CDR-L3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:262, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:265, and SEQ ID NO:266.

14. The bispecific antigen binding molecule of any one of claims 1 to 3, 9 or 11 to 13, wherein each of the moieties capable of specific binding to 4-1BB 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:267, SEQ ID NO: 269, SEQ ID NO:271, SEQ ID NO:273, and SEQ ID NO:275, 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 selected from the group consisting of SEQ ID NO:268, SEQ ID NO: 270, SEQ ID NO:272, SEQ ID NO:274, and SEQ ID NO:276.

15. The bispecific antigen binding molecule of any one of claims 1 to 3, 9 or 11 to 14, wherein each of the moieties capable of specific binding to 4-1BB comprises

- (i) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:267 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:268,

- (ii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:269 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:270,
- (iii) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:271 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:272,
- (iv) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:273 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:274, or
- (v) a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:275 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:276.

16. The bispecific antigen binding molecule of any one of claims 1 to 3, 9 or 11 to 15, wherein

- (i) each of the moieties capable of specific binding to 4-1BB 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 the amino acid sequence selected from the group consisting of SEQ ID NO:267, SEQ ID NO: 269, SEQ ID NO:271, SEQ ID NO:273, and SEQ ID NO:275 and a light chain variable region 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:268, SEQ ID NO: 270, SEQ ID NO:272, SEQ ID NO:274, and SEQ ID NO:276, and
- (ii) the moiety capable of specific binding to FAP 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 the amino acid sequence of SEQ ID NO:51 or SEQ ID NO:53 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:52 or SEQ ID NO:54.

17. The bispecific antigen binding molecule of any one of claims 1 to 3 or 9, wherein the costimulatory TNF receptor family member is GITR.

18. The bispecific antigen binding molecule of any one of claims 1 to 3 or 9 or 17, wherein 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:357.

19. The bispecific antigen binding molecule of any one of claims 1 to 3, 9 or 17 or 18, comprising four moieties capable of specific binding to GITR, wherein each of said moieties comprises a VH domain comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO:371, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:372 and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:373, and a VL domain comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO:374, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:375 and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:376.

20. The bispecific antigen binding molecule of any one of claims 1 to 3, 9 or 17 to 19, wherein each of the moieties capable of specific binding to GITR 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 of SEQ ID NO:383, 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:384.

21. The bispecific antigen binding molecule of any one of claims 1 to 3, 9 or 17 to 20, wherein each of the moieties capable of specific binding to GITR comprises a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:383 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:384.

22. The bispecific antigen binding molecule of any one of claims 1 to 3, 9 or 17 to 21, wherein

- (i) each of the moieties capable of specific binding to GITR 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 the amino acid sequence of SEQ ID NO:383, and a light chain variable region 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:384, and
- (ii) the moiety capable of specific binding to FAP 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 the amino acid sequence of SEQ ID NO:51 or SEQ ID NO:53 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:52 or SEQ ID NO:54.

23. The bispecific antigen binding molecule of any one of claims 1 to 22, wherein the four moieties capable of specific binding to a costimulatory TNF receptor family member are Fab fragments and each two thereof are fused to each other, optionally via a peptide linker.

24. The bispecific antigen binding molecule of any one of claims 1 to 23, wherein a first Fab fragment capable of specific binding to a costimulatory TNF receptor family member is fused at the C-terminus of the CH1 domain to the VH domain of a second Fab fragment capable of specific binding to a costimulatory TNF receptor family member and a third Fab fragment capable of specific binding to a costimulatory TNF receptor family member is fused at the C-terminus of the CH1 domain to the VH domain of a fourth Fab fragment capable of specific binding to a costimulatory TNF receptor family member, optionally via a peptide linker.

25. The bispecific antigen binding molecule of any one of claims 1 to 24, wherein in the Fab fragments capable of specific binding to a costimulatory TNF receptor family member in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat EU Index), and in the constant domain CH1 the amino acids at positions 147 and 213 are substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

26. The bispecific antigen binding molecule of any one of claims 1 to 25, wherein the bispecific antigen binding molecule is tetravalent for the costimulatory TNF receptor family member and monovalent for the target cell antigen.

27. The bispecific antigen binding molecule of any one of claims 1 to 26, wherein the moiety capable of specific binding to a target cell antigen comprises a VH and VL domain and wherein the VH domain is connected via a peptide linker to the C-terminus of the first subunit of the Fc domain and the VL domain is connected via a peptide linker to the C-terminus of the second subunit of the Fc domain.

28. The bispecific antigen binding molecule of any one of claims 1 to 25, wherein the bispecific antigen binding molecule is tetravalent for the costimulatory TNF receptor family member and bivalent for the target cell antigen.

29. The bispecific antigen binding molecule of any one of claims 1 to 25 or 28, wherein the two moieties capable of specific binding to a target cell antigen are Fab fragments or crossover Fab fragments.

30. The bispecific antigen binding molecule of any one of claims 1 to 25 or 28 to 29, wherein each of the Fab fragments or crossover Fab fragments capable of specific binding to a target cell antigen is fused at the N-terminus of the VH or VL domain via a peptide linker to the C-terminus of one of the subunits of the Fc domain.

31. The bispecific antigen binding molecule of any one of claims 1 to 25 or 28 to 30, wherein the two moieties capable of specific binding to a target cell antigen are VH-VL

crossover Fab fragments and are each fused at the N-terminus of the VL domain via a peptide linker to the C-terminus of one of the subunits of the Fc domain.

32. The bispecific antigen binding molecule of any one of claims 1 to 31, wherein the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

33. A polynucleotide encoding the bispecific antigen binding molecule of any one of claims 1 to 32.

34. A pharmaceutical composition comprising a bispecific antigen binding molecule of any one of claims 1 to 32, and at least one pharmaceutically acceptable excipient.

35. The bispecific antigen binding molecule of any one of claims 1 to 32, or the pharmaceutical composition of claim 34, for use as a medicament.

36. The bispecific antigen binding molecule of any one of claims 1 to 32, or the pharmaceutical composition of claim 34, 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.

37. The bispecific antigen binding molecule of any one of claims 1 to 32, or the pharmaceutical composition of claim 34, for use in the treatment of cancer.

38. The bispecific antigen binding molecule of any one of claims 1 to 32, or the pharmaceutical composition of claim 34, 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.

39. Use of the bispecific antigen binding molecule of any one of claims 1 to 34, or the pharmaceutical composition of claim 36 in the manufacture of a medicament for the treatment of cancer.