

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 at least one moiety capable of specific binding to a costimulatory TNF receptor family member, i.e. at least one antigen binding site that targets 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 also provides novel antibodies capable of specific binding to a costimulatory TNF receptor family member. In comparison to known antibodies to costimulatory TNF receptors these antibodies have properties that are advantageous for incorporating them into bispecific antigen binding molecules in combination with moieties capable of specific binding to a target cell antigen.

The disclosure provides a bispecific antigen binding molecule, comprising

- (a) at least one moiety 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 a particular embodiment, the bispecific antigen binding molecule comprises (a) at least one moiety 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 and 4-1BB, (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 particular aspect, the bispecific antigen binding molecule comprises (a) at least one moiety capable of specific binding to a OX40, (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. 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,

- (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 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:39.

Furthermore, provided is a bispecific antigen binding molecule, comprising at least one moiety capable of specific binding to 4-1BB, 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:40 and SEQ ID NO:41,
 - (ii) a CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:43, and
 - (iii) a CDR-H3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47 and SEQ ID NO:48
- and a VL domain comprising
- (iv) a CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:49 and SEQ ID NO:50,
 - (v) a CDR-L2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:51 and SEQ ID NO:52, and

(vi) a CDR-L3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56 and SEQ ID NO:57.

In another embodiment, the disclosure provides a bispecific antigen binding molecule, wherein the moiety 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:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64 and SEQ ID NO:66 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:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65 and SEQ ID NO:67.

Particularly, a bispecific antigen binding molecule is provided, wherein the moiety capable of specific binding to 4-1BB 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.

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

- (a) at least one moiety 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., wherein the moiety capable of specific binding to a costimulatory TNF receptor family member is a Fab fragment. In one aspect, if the bispecific antigen binding molecule , comprises more than one moiety capable of specific binding to a costimulatory TNF receptor family member, all moieties capable of specific binding to a costimulatory TNF receptor family member are Fab fragments.

In another embodiment, the bispecific antigen binding molecule comprises (a) at least one moiety 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:68 and SEQ ID NO:69,
- (ii) a CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:70 and SEQ ID NO:71, and
- (iii) a CDR-H3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:72 and SEQ ID NO:73,

and a VL domain comprising

- (iv) a CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:74 and SEQ ID NO:75,
- (v) a CDR-L2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:76 and SEQ ID NO:77, and
- (vi) a CDR-L3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:78 and SEQ ID NO:79.

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

- (i) 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 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:80 or SEQ ID NO:82 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:81 or SEQ ID NO:83.

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

(i) the moiety capable of specific binding to OX40 comprises 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. In another aspect, the disclosure provides a bispecific antigen binding molecule, wherein

(i) the moiety 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 of SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64 or SEQ ID NO:66 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:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65 or SEQ ID NO:67 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:80 or SEQ ID NO:82 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:81 or SEQ ID NO:83.

In a further embodiment, provided is a bispecific antigen binding molecule, wherein said molecule comprises

- (a) a first Fab fragment capable of specific binding to a costimulatory TNF receptor family member,
- (b) a second Fab fragment 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 bispecific antigen binding molecule is a human, a humanized or a chimeric antibody. In particular, the Fc domain is a human IgG domain, particularly an IgG1 Fc domain or an IgG4 Fc domain.

In a further embodiment, provided is a bispecific antigen binding molecule, wherein the Fc domain comprises one or more amino acid substitution that reduces the binding affinity of the antibody to an Fc receptor and/or effector function. In particular, the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

In a further embodiment, provided is a bispecific antigen binding molecule, wherein the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain. In a particular embodiment, the disclosure provides a bispecific antigen binding molecule, 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. More 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 particular the disclosure provides a bispecific antigen binding molecule, comprising
 (a) two moieties capable of specific binding to a costimulatory TNF receptor family member,
 (b) two moieties 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.

Thus, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule is bivalent both for the costimulatory TNF receptor family member and for the target cell antigen.

In a particular embodiment, the bispecific antigen binding molecule comprises
 (a) two light chains and two heavy chains of an antibody comprising two Fab fragments capable of specific binding to a costimulatory TNF receptor family member and the Fc domain, and
 (b) two additional Fab fragments capable of specific binding to a target cell antigen, wherein said additional Fab fragments are each connected via a peptide linker to the C-terminus of the heavy chains of (a). More particularly, the two additional Fab fragments capable of specific binding to a target cell antigen are cross-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).

In one embodiment, the two Fab fragments capable of specific binding to a costimulatory TNF receptor family member are two Fab fragments capable of specific binding to OX40 or 4-1BB and the two additional Fab fragments capable of specific binding to a target cell antigen are cross-Fab fragments capable of specific binding to FAP.

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

- (a) two moieties capable of specific binding to a costimulatory TNF receptor family member,
- (b) 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.

Thus, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule is bivalent for the costimulatory TNF receptor family member and monovalent for the target cell antigen.

In a particular embodiment, the bispecific antigen binding molecule comprises

- (a) two light chains and two heavy chains of an antibody comprising two Fab fragments capable of specific binding to a costimulatory TNF receptor family member 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.

In another aspect, the disclosure provides an antibody that specifically binds to OX40, wherein said antibody 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,
- (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,
- (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 bispecific antigen binding molecule as described herein. The disclosure, in another aspect, provides a polynucleotide encoding the 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 aspects 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 a bispecific antigen binding molecule as described herein or an 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 bispecific antigen binding molecule as described herein, or the 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 antibody 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,
- (v) in delaying progression of cancer, or
- (vi) in prolonging the survival of a patient suffering from 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 bispecific antigen binding molecule as described herein, or the 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 bispecific antigen binding molecule as described herein, or the 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 aspect, 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 aspect, the disease is cancer. In any of the above aspects the individual is a mammal, particularly a human.

The disclosure, in another aspect, provides use of the bispecific antigen binding molecule as described herein, or the 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.

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.

Figure 2 shows the binding of anti-OX40 antibodies to activated human CD4⁺ and CD8⁺ T cells. OX40 is not expressed on resting human PBMCs (Figure 2A and 2C). After activation of human PBMCs OX40 is up-regulated on CD4⁺ and CD8⁺ T cells (Figure 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.

Claims

1. A bispecific antigen binding molecule, comprising

- (a) at least one moiety capable of specific binding to a OX40,
- (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.

2. The bispecific antigen binding molecule of claim 1, 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.

3. The bispecific antigen binding molecule of claim 1 or 2, 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.

4. The bispecific antigen binding molecule of claim 1 or 2, 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,
- (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,
- (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.

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 is a Fab fragment.

6. The bispecific antigen binding molecule of any one of claims 1 to 5, 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.

7. The bispecific antigen binding molecule of any one of claims 1 to 6, wherein the target cell antigen is Fibroblast Activation Protein (FAP).

8. The bispecific antigen binding molecule of any one of claims 1 to 7, 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:68 and SEQ ID NO:69,

(ii) a CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:70 and SEQ ID NO:71, and

(iii) a CDR-H3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:72 and SEQ ID NO:73,

and a VL domain comprising

(iv) a CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:74 and SEQ ID NO:75,

(v) a CDR-L2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:76 and SEQ ID NO:77, and

(vi) a CDR-L3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:78 and SEQ ID NO:79.

9. The bispecific antigen binding molecule of any one of claims 1 to 6, wherein

(i) 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 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 VL 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:80 or SEQ ID NO:82 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 the amino acid sequence of SEQ ID NO:81 or SEQ ID NO:83.

10. The bispecific antigen binding molecule of any one of claims 1 to 4 or 9, wherein

(i) the moiety capable of specific binding to OX40 comprises 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.

11. The bispecific antigen binding molecule of any one of claims 1 to 10, wherein said molecule comprises

- (a) a first Fab fragment capable of specific binding to OX40,
- (b) a second Fab fragment 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.

12. The bispecific antigen binding molecule of any one of claims 1 to 11, wherein the Fc domain is an IgG, particularly an IgG1 Fc domain or an IgG4 Fc domain.

13. The bispecific antigen binding molecule of any one of claims 1 to 12, wherein the Fc domain comprises one or more amino acid substitution that reduces the binding affinity of the antibody to an Fc receptor and/or effector function.

14. The bispecific antigen binding molecule of any one of claims 1 to 13, wherein the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

15. The bispecific antigen binding molecule of any one of claims 1 to 14, wherein the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain.

16. The bispecific antigen binding molecule of any one of claims 1 to 15, 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.

17. The bispecific antibody of any one of claims 1 to 16, 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).

18. The bispecific antigen binding molecule of any one of claims 1 to 17, comprising

- (a) two moieties capable of specific binding to OX40,
- (b) two moieties 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.

19. The bispecific antigen binding molecule of claim 18, wherein the bispecific antigen binding molecule is bivalent both for OX40 and for the target cell antigen.

20. The bispecific antigen binding molecule of any one of claims 1 to 17, 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 a target cell antigen, wherein said additional Fab fragments are each connected via a peptide linker to the C-terminus of the heavy chains of (a).

21. The bispecific antigen binding molecule of claim 20, wherein the two additional Fab fragments capable of specific binding to a target cell antigen 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).

22. The bispecific antigen binding molecule of any one of claims 1 to 17, comprising

- (a) two moieties capable of specific binding to OX40,
- (b) 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.

23. The bispecific antigen binding molecule of claim 22, wherein the bispecific antigen binding molecule is bivalent for OX40 and monovalent for the target cell antigen.

24. The bispecific antigen binding molecule of claims 1 to 17 or 22 or 23, 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.

25. A polynucleotide encoding the bispecific antigen binding molecule of any one of claims 1 to 24.

26. An antibody that specifically binds to OX40, wherein said antibody 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,
- (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,
- (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.

27. A polynucleotide encoding the antibody of claim 26.

28. A pharmaceutical composition comprising a bispecific antigen binding molecule of any one of claims 1 to 24 or an antibody of claim 26 and at least one pharmaceutically acceptable excipient.

29. The bispecific antigen binding molecule of any one of claims 1 to 24, or the antibody of claim 26 or the pharmaceutical composition of claim 28, for use as a medicament.

30. The bispecific antigen binding molecule of any one of claims 1 to 24, or the antibody of claim 26, or the pharmaceutical composition of claim 28, 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.

31. The bispecific antigen binding molecule of any one of claims 1 to 24, or the antibody of claim 26, or the pharmaceutical composition of claim 28, for use in the treatment of cancer.

32. The bispecific antigen binding molecule of any one of claims 1 to 24, or the antibody of claim 26, or the pharmaceutical composition of claim 28, 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.

33. Use of the bispecific antigen binding molecule of any one of claims 1 to 24, or the antibody of claim 26, or the pharmaceutical composition of claim 28, in the manufacture of a medicament for the inhibition of growth of tumor cells or for the treatment of cancer.
