

(e.g. cytokine secretion). Conversely, OX40 signaling leads to functional inhibition and loss of regulatory T cells. OX40 agonism counterbalances TGF- β effects, (e.g. impedes FoxP3 induction) and lowers IL-10 secretion. In murine tumor models, OX40 engagement by an agonist anti-OX40 antibody can promote anti-tumor T-cell responses, tumor shrinkage and reproducible abscopal effects. Monotherapy efficacy of OX40 agonists was in general low, but strong anti-tumor efficacy was achieved in combination with immunogenic treatments (chemotherapy, radiation and vaccination), check point inhibitors (PD-1, CTLA-4) and other costimulatory agonists such as 4-1BB, ICOS or GITR.

Fibroblast activation protein- α (FAP) is a serine protease highly expressed on the cell surface of cancer-associated stroma cells of > 90% of human epithelial malignancies, on reticular fibroblasts, which are in the T cell priming zones of the lymph nodes, and can be found on activated fibroblasts in normal tissues. High prevalence in various cancer indications allows its usage as targeting moiety for drugs that should accumulate within the tumor environment.

One means to restore OX40 co-stimulation specifically in the tumor microenvironment are bispecific antibodies comprised of at least one antigen binding domain for fibroblast activating protein (FAP) in the tumor stroma, and at least one antigen binding domain for OX40. For example, such bispecific antibodies have been described in WO 2017/055398 A2 and WO 2017/060144 A1. Crosslinking and surface immobilization of such bispecific molecules by cell surface FAP creates a highly agonistic matrix for OX40 positive T cells, where it supports NF κ B mediated effector functions and can replace ligation by OX40 Ligand. High FAP expression is reported for a plethora of human tumor indications, either on tumor cells themselves or on immune suppressive cancer associated fibroblasts (CAFs). There is thus a need for improved FAP-targeted OX40 bispecific antibodies with excellent pharmacological properties such as better shelf-life, less immunogenicity and with less unspecific interactions such as hypersensitivity reactions or uncontrolled cytokine release.

SUMMARY OF THE INVENTION

This inventiondisclosure relates to new bispecific antigen binding molecules capable of specific binding to OX40 and Fibroblast Activation Protein (FAP) with improved properties, wherein the OX40 co-stimulation is provided by cross-linking through FAP expressed on tumor stroma cells and potentially also through FAP intermediately expressed in secondary lymphoid tissues. The antigen binding molecules of this inventiondisclosure are thus able to trigger OX40 not only effectively, but also very selectively at the desired site while overcoming the need for Fc γ R cross-linking thereby reducing side effects. The

new bispecific antigen binding molecules are characterized by comprising a new FAP antigen binding domain fused to the C-terminus of the Fc domain and improved pharmacokinetic properties.

The bispecific antigen binding molecules of the present ~~invention~~disclosure combine at least two antigen binding domains capable of specific binding to the costimulatory TNF receptor family member OX40, with an antigen binding domain targeting Fibroblast Activation Protein (FAP) comprising a new murine anti-human FAP clone 212 and humanized variants thereof. These bispecific antigen binding molecules are OX40 agonists and advantageous as they will preferably activate costimulatory OX40 receptors close to the tumor site where FAP is expressed because they are able to bind to FAP with high affinity. The molecules are further designed to have an advantageous pharmacokinetic profile to optimize the treatment regimen resulting in an enhanced safety to efficacy balance.

In one aspect, the ~~invention~~disclosure provides a bispecific antigen binding molecule, comprising

- (a) at least two antigen binding domains capable of specific binding to OX40,
- (b) an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP) comprising a heavy chain variable region (V_H FAP) comprising
 - (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_L FAP) comprising
 - (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and
 - (c) a Fc region composed of a first and a second subunit capable of stable association.

In one ~~aspect~~example, the Fc region comprises one or more amino acid substitution that reduces the binding affinity of the antibody to an Fc receptor and/or effector function.

In one further ~~aspect~~example, provided is a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising an amino acid sequence that is at least about 90% identical to the amino acid sequence of SEQ ID NO:9, and a light chain variable region (V_L FAP) comprising an amino acid sequence that is at least about 90% identical to the amino acid sequence of SEQ ID NO:10. In one ~~aspect~~example, the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising

the amino acid sequence of SEQ ID NO:9, and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:10.

In another [aspectexample](#), the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19 and SEQ ID NO:20, and a light chain variable region (V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26. In one [aspectexample](#), the antigen binding domain capable of specific binding to FAP comprises (a) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21, (b) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:16 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21, (c) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:16 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:22, or (d) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:19 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:25. Particularly, the antigen binding domain capable of specific binding to FAP comprises (a) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21.

In one [aspectexample](#), the antigen binding domains capable of specific binding to OX40 bind to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:1.

In a further [aspectexample](#), provided is a bispecific antigen binding molecule, wherein the antigen binding domains capable of specific binding to OX40 (each) comprise (a) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:27, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:28, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:29, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:32, or (b) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:35, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:36, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:37, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino

acid sequence of SEQ ID NO:38, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:39, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:40, or (c) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:43, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:44, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:45, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:47, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:48, or (d) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:51, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:52, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:53, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:54, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:55, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:56.

15 In one [aspectexample](#), the antigen binding domains capable of specific binding to OX40 (each) comprise a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:27, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:28, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:29, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:32. In a further [aspectexample](#), the antigen binding domains capable of specific binding to OX40 comprise a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:35, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:36, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:37, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:38, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:39, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:40. In another [aspectexample](#), the antigen binding domains capable of specific binding to OX40 comprise a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:43, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:44, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:45, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:47, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:48. In yet another [aspectexample](#), the antigen binding domains capable of specific binding to OX40 comprise a heavy chain variable region (V_HOX40) comprising (i)

CDR-H1 comprising the amino acid sequence of SEQ ID NO:51, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:52, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:53, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:54, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:55, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:56.

In one [aspectexample](#), provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domains capable of specific binding to OX40 (each) comprise

(i) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:33 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34, or

(ii) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:41 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:42, or

(iii) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:49 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:50, or

(iv) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:57 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:58.

In one [aspectexample](#), provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domains capable of specific binding to OX40 (each) comprise a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:33 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34. In another [aspectexample](#), provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:41 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:42. In a further [aspectexample](#), provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:49 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:50. In yet as further [aspectexample](#), provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID

NO:57 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:58.

In a particular [aspectexample](#), provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domains capable of specific binding to OX40 (each) comprise a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:41 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:42.

In another [aspectexample](#), provided is a bispecific antigen binding molecule as defined herein before, the antigen binding domains capable of specific binding to OX40 comprise

(i) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:59 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34, or

(ii) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:60 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34, or

(iii) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:61 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34.

In one [aspectexample](#), provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:59 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34. In one [aspectexample](#), the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:60 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34. In a further [aspectexample](#), the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:61 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34.

In another [aspectexample](#), the bispecific antigen binding molecule is a humanized or a chimeric antibody, in particular a humanized antibody. In a further [aspectexample](#), the bispecific antigen binding molecule comprises an IgG Fc region, particularly an IgG1 Fc region or an IgG4 Fc region. In particular, the Fc region comprises one or more amino acid substitution that reduces the binding affinity of the antibody to an Fc receptor and/or effector function. In one particular [aspectexample](#), provided is a bispecific antigen binding molecule, wherein the Fc

region is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

In another [aspectexample](#), provided is a bispecific antigen binding molecule as defined herein before, wherein the first subunit of the Fc region comprises knobs and the second subunit of the Fc region comprises holes according to the knobs into holes method. In particular, provided is a bispecific antigen binding molecule, wherein the first subunit of the Fc region comprises the amino acid substitutions S354C and T366W (numbering according to Kabat EU index) and the second subunit of the Fc region comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index). In yet another [aspectexample](#), the Fc region is of murine origin and the first subunit of the Fc region comprises the amino acid substitutions K392D and K409D (numbering according to Kabat EU index) and the second subunit of the Fc region comprises the amino acid substitutions E356K and D399K (numbering according to Kabat EU index). More particularly, provided is a bispecific antigen binding molecule, wherein the first subunit of the Fc region comprises the amino acid substitutions S354C and T366W (numbering according to Kabat EU index) and the second subunit of the Fc region comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index).

In a further [aspectexample](#), provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule comprises

- (a) at least two Fab fragments capable of specific binding to OX40 each connected to the N-terminus of one of subunits of the Fc region, and
- (b) one cross-Fab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region, and
- (c) the Fc region composed of a first and a second subunit capable of stable association.

Thus, provided is a bispecific antigen binding molecule that provides bivalent binding towards OX40 and monovalent binding towards FAP (2+1 format). In particular, the bispecific antigen binding molecule comprises one cross-Fab fragment capable of specific binding to FAP, wherein the VH-Ckappa chain of the cross-fab fragment capable of specific binding to FAP is fused to the C-terminus of one of subunits of the Fc region. In one particular [aspectexample](#), the VH-Ckappa chain of the cross-fab fragment capable of specific binding to FAP is fused to the C-terminus of one of first subunit of the Fc region comprising the amino acid substitutions S354C and T366W (numbering according to Kabat EU index), i.e. to the knob chain.

In one [aspectexample](#), provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule consists of

- (aa) a first Fab fragment capable of specific binding to OX40,
- (ab) a second Fab fragment capable of specific binding to OX40,
- 5 (b) a cross-Fab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region, and
- (c) the Fc region composed of a first and a second subunit capable of stable association, wherein the first Fab fragment (aa) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the first subunit and the second Fab fragment (ab) is fused at the
- 10 C-terminus of the VH-CH1 chain to the N-terminus of the second subunit.

Thus, provided is a bispecific antigen binding molecule that provides bivalent binding towards OX40 and monovalent binding towards FAP (2+1 format).

In another [aspectexample](#), provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule consists of

- 15 (aa) a first Fab fragment capable of specific binding to OX40,
- (ab) a second Fab fragment capable of specific binding to OX40,
- (ac) a third Fab fragment capable of specific binding to OX40,
- (b) a cross-Fab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region, and
- 20 (c) the Fc region composed of a first and a second subunit capable of stable association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the first subunit, and the third Fab fragment (ac) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the
- 25 second subunit.

Thus, provided is a bispecific antigen binding molecule that provides trivalent binding towards OX40 and monovalent binding towards FAP (3+1 format).

In one [aspectexample](#), provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule consists of

- 30 (aa) a first Fab fragment capable of specific binding to OX40,
- (ab) a second Fab fragment capable of specific binding to OX40,
- (ac) a third Fab fragment capable of specific binding to OX40,
- (b) a cross-Fab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region, and

(c) the Fc region composed of a first and a second subunit capable of stable association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the second subunit of the Fc region comprising the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index), and the third Fab fragment (ac) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit comprising the amino acid substitutions S354C and T366W (numbering according to Kabat EU index). In particular, the VH-Ckappa chain of the cross-fab fragment capable of specific binding to FAP is fused to the C-terminus of the first subunit of the Fc region comprising the amino acid substitutions S354C and T366W (numbering according to Kabat EU index).

In one aspectexample, provided is a bispecific antigen binding molecule comprising (a) two heavy chains, one heavy chain comprising two VH-CH1 chains of a Fab fragment capable of specific binding to OX40 that are connected to each other, optionally by a peptide linker, and a Fc region subunit and one heavy chain comprising a VH-CH1 chain of a Fab fragment capable of specific binding to OX40 and a Fc region subunit, (b) three light chains, each light chain comprising a VL and Ckappa domain of a Fab fragment capable of specific binding to OX40, and (c) a cross-Fab fragment capable of specific binding to FAP comprising a VL-CH1 light chain and a VH-Ckappa chain, wherein the VH-Ckappa chain is connected to the C-terminus of one of the two heavy chains of (a), optionally by a peptide linker.

In one aspectexample, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule consists of

- (aa) a first Fab fragment capable of specific binding to OX40,
- (ab) a second Fab fragment capable of specific binding to OX40,
- (ac) a third Fab fragment capable of specific binding to OX40,
- (ad) a fourth Fab fragment capable of specific binding to OX40,
- (b) a cross-Fab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region, and
- (c) the Fc region composed of a first and a second subunit capable of stable association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the first subunit, and the fourth Fab fragment (ad) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the third Fab fragment (ac), which is in turn fused at its C-terminus to the N-terminus of the second subunit.

Thus, provided is a bispecific antigen binding molecule that provides tetravalent binding towards OX40 and monovalent binding towards FAP (4+1 format). In one particular ~~aspect~~example, the VH-Ckappa chain of the cross-fab fragment capable of specific binding to FAP is fused to the C-terminus of the first subunit of the Fc region comprising the amino acid substitutions S354C and T366W (numbering according to Kabat EU index).

In one ~~aspect~~example, provided is a bispecific antigen binding molecule, wherein each of the two heavy chains comprises two VH-CH1 chains of a Fab fragment capable of specific binding to OX40 that are fused to each other, optionally by a peptide linker. Thus, in one ~~aspect~~example, the ~~invention~~disclosure provides a bispecific antigen binding molecule comprising (a) two heavy chains, each heavy chain comprising two VH-CH1 chains of a Fab fragment capable of specific binding to OX40 that are connected to each other, optionally by a peptide linker, and a Fc region subunit, (b) four light chains, each light chain comprising a VL and Ckappa domain of a Fab fragment capable of specific binding to OX40, and (c) a cross-Fab fragment capable of specific binding to FAP comprising a VL-CH1 light chain and a VH-Ckappa chain, wherein the VH-Ckappa chain is connected to the C-terminus of one of the two heavy chains of (a), optionally by a peptide linker.

According to another aspect of the ~~invention~~disclosure, there is provided isolated nucleic acid encoding ~~a~~the bispecific antigen binding molecule as described herein before. The ~~invention~~disclosure further provides a vector, particularly an expression vector, comprising the isolated nucleic acid of the ~~invention~~disclosure, and a host cell comprising the isolated nucleic acid or the expression vector of the ~~invention~~disclosure. In another aspect, the present disclosure provides an expression vector comprising the isolated nucleic acid as described herein. In another aspect, the present disclosure provides a host cell comprising isolated nucleic acid as described herein or the expression vector as described herein. In some ~~aspect~~examples, the host cell is a eukaryotic cell, particularly a mammalian cell. In another aspect, provided is a method of producing a bispecific antigen binding molecule ~~as described herein before~~, comprising culturing the host cell as described above under conditions suitable for the expression of the bispecific antigen binding molecule, and isolating the bispecific antigen binding molecule. The ~~invention~~disclosure also encompasses the bispecific antigen binding molecule that specifically binds to OX40 and to FAP produced by the method of the ~~invention~~disclosure.

~~The invention~~In another aspect, the disclosure further provides a pharmaceutical composition comprising ~~a~~the bispecific antigen binding molecule as described herein before and a pharmaceutically acceptable carrier. In one ~~aspect~~example, the pharmaceutical composition comprises an additional therapeutic agent.

- 5 Also encompassed by the ~~invention~~disclosure is the bispecific antigen binding molecule or the antibody as described herein before, or the pharmaceutical composition comprising the bispecific antigen binding molecule, for use as a medicament. In another aspect, the present disclosure provides the bispecific antigen binding molecule as described herein, or the pharmaceutical composition as described herein, for use as a medicament.

In ~~one another~~ aspect, provided is ~~a the~~ bispecific antigen binding molecule as described herein before, or the pharmaceutical composition of the ~~invention~~disclosure, for use

- (i) in inducing immune stimulation,
- 5 (ii) in stimulating tumor-specific T cell response,
- (iii) in causing apoptosis of tumor cells,
- (iv) in the treatment of cancer,
- (v) in delaying progression of cancer,
- (vi) in prolonging the survival of a patient suffering from cancer,
- 10 (vii) in the treatment of infections.

In a specific aspect, provided is the bispecific antigen binding molecule as described herein before, or the pharmaceutical composition of the ~~invention~~disclosure, for use in the treatment of cancer. ~~In another specific aspect, the invention~~The disclosure provides the bispecific antigen binding molecule as described herein before for use in the treatment of

15 cancer, wherein the bispecific antigen binding molecule is for administration in combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy. In another aspect, the present disclosure provides the bispecific antigen binding molecule as described herein, or the pharmaceutical composition as described herein, for use in the treatment of cancer, wherein the bispecific antigen binding molecule

20 or pharmaceutical composition is to be administered in combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy. In ~~one aspect, provided is~~The present disclosure provides a bispecific agonistic OX40 antigen binding molecule or a pharmaceutical composition for use in the treatment of cancer, wherein the bispecific agonistic OX40 antigen binding molecule is for administration in

25 combination with a T-cell activating anti-CD3 bispecific antibody, for example an anti-CEA/anti-CD3 bispecific antibody. In another aspect, the present disclosure provides the bispecific antigen binding molecule as described herein, or the pharmaceutical composition as described herein, for use in the treatment of cancer, wherein the bispecific agonistic OX40 antigen binding molecule is to be administered in combination with a T-cell

30 activating anti-CD3 bispecific antibody. In one further ~~aspect~~example, the bispecific antigen binding molecule as described herein is for use in the treatment of cancer, wherein the bispecific antigen binding molecule is for administration in combination with an agent blocking PD-L1/PD-1 interaction such as PD-L1 antibody, for example atezolizumab, or a PD-1 antibody, for example nivolumab or pembrolizumab. In another ~~aspect~~example,

provided is the bispecific antigen binding molecule as described herein before or the pharmaceutical composition of the ~~invention~~disclosure, for use in up-regulating or prolonging cytotoxic T cell activity.

~~In a further aspect, the invention~~The present disclosure provides a method of
5 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 ~~invention~~disclosure, to inhibit the growth of the tumor cells. ~~In another aspect, the invention~~The present disclosure provides a method of treating or delaying cancer in an individual comprising administering to the
10 individual an effective amount of the bispecific antigen binding molecule as described herein before, or the pharmaceutical composition of the ~~invention~~disclosure.

Also provided is the use of 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 ~~invention~~disclosure in a pharmaceutically acceptable form. In a specific ~~aspect~~example, the disease is cancer. In another aspect, the present disclosure provides use of the bispecific antigen binding molecule as described herein, or the pharmaceutical composition as described herein, in the manufacture of a medicament for the treatment of cancer. In any of the above aspectsWhere mentioned above, the individual is a mammal, particularly a human.

BRIEF DESCRIPTION OF THE DRAWINGS

- Figures 1A to E** show schematic representations of bispecific antigen binding molecules which specifically bind to human OX40 and to FAP. **Fig. 1A** shows a schematic representation of a bispecific FAP-OX40 antibody in a 4+1 format consisting of four OX40 binding Fab fragments combined with one FAP (1G1a) binding moiety as crossFab fragment, wherein the VH-Ckappa chain is fused at the C-terminus of the Fc knob chain (tetravalent for OX40 and monovalent for FAP). **Fig. 1B** shows a schematic representation of a bispecific FAP-OX40 antibody in a 3+1 format consisting of three OX40 binding Fab fragments combined with one FAP (1G1a) binding moiety as crossFab fragment, wherein the VH-Ckappa chain is fused at the C-terminus of the Fc knob chain (trivalent for OX40 and monovalent for FAP). The arm comprising two OX40 binding Fab fragments fused to each other is on Fc knob chain. **Fig. 1C** shows a schematic representation of a bispecific FAP-OX40 antibody in a 3+1 format consisting of three OX40 binding Fab fragments combined with one FAP (1G1a) binding moiety as crossFab fragment, wherein the VH-Ckappa chain is fused at the C-terminus of the Fc knob chain (trivalent for OX40 and monovalent for FAP). The arm comprising two OX40 binding Fab fragments fused to each other is on Fc hole chain. **Fig. 1D** shows a schematic representation of a bispecific FAP-OX40 antibody in a 2+1 format consisting of two OX40 binding Fab fragments combined with one FAP (1G1a) binding moiety as crossFab fragment, wherein the VH-Ckappa chain is fused at the C-terminus of the Fc knob chain (bivalent for OX40 and monovalent for FAP). **Fig. 1E** shows a schematic representation of a bispecific FAP-OX40 antibody P1AD4524 in a 4+1 format consisting of four OX40 binding Fab fragments combined with one FAP (4B9) binding moiety as VH and VL domain, wherein the VL domain is fused at the C-terminus of the Fc knob chain and the VH domain is fused at the C-terminus of the Fc hole chain (tetravalent for OX40 and monovalent for FAP). The black point symbolizes knob-into-hole mutations.
- Figures 2A to 2F** show the cellular binding of bispecific antigen binding molecules comprising OX40 clone 49B4 in different formats. The FAP antigen binding domain H212 is the humanized version of FAP clone 212 that is called FAP (1G1a) herein. Human FAP negative tumor cells (A549- NLR)(**Fig. 2F**), FAP positive fibroblasts (NIH/3T3-huFAP-clone 19) (**Fig. 2E**), OX40 positive activated PBMC (activated CD4 and CD8 T cells, **Fig.**

Claims

1. A bispecific antigen binding molecule, comprising
 - (a) at least two antigen binding domains capable of specific binding to OX40,
 - 5 (b) an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP) comprising a heavy chain variable region (V_H FAP) comprising
 - (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid
 - 10 sequence of SEQ ID NO:5, and a light chain variable region (V_L FAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and
 - 15 (c) a Fc region composed of a first and a second subunit capable of stable association.

2. The bispecific antigen binding molecule of claim 1, wherein the Fc region comprises one or more amino acid substitution that reduces the binding affinity of the antibody to an Fc receptor and/or effector function.

3. The bispecific antigen binding molecule of claims 1 or 2, wherein the antigen
 - 20 binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising an amino acid sequence that is at least about 90% identical to the amino acid sequence of SEQ ID NO:9, and a light chain variable region (V_L FAP) comprising an amino acid sequence that is at least about 90% identical to the amino acid sequence of SEQ ID NO:10.

4. The bispecific antigen binding molecule of any one of claims 1 to 3, wherein the
 - 25 antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19 and SEQ ID NO:20, and
 - 30 a light chain variable region (V_L FAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26.

5. The bispecific antigen binding molecule of any one of claims 1 to 4, wherein the antigen binding domain capable of specific binding to FAP comprises

- (a) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21, or
- (b) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:16 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21, or
- (c) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:16 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:22, or
- (d) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:19 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:25.

6. The bispecific antigen binding molecule of any one of claims 1 to 4, wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21.

7. The bispecific antigen binding molecule of any one of claims 1 to 6, wherein the antigen binding domain capable of specific binding to OX40 comprises
- (i) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:27, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:28, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:29, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:32, or
 - (ii) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:35, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:36, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:37, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:38, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:39, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:40, or
 - (iii) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:43, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:44, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:45, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, (v) CDR-L2 comprising the amino acid

sequence of SEQ ID NO:47, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:48, or

- (iv) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:51, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:52, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:53, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:54, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:55, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:56.

- 10 8. The bispecific antigen binding molecule of any one of claims 1 to 7, wherein the antigen binding domain capable of specific binding to OX40 comprises

(i) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:33 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34, or

- 15 (ii) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:41 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:42, or

- (iii) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:49 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:50, or

(iv) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:57 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:58.

- 25 9. The bispecific antigen binding molecule of any one of claims 1 to 7, wherein the antigen binding domain capable of specific binding to OX40 comprises

(i) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:59 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34, or

- 30 (ii) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:60 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34, or

(iii) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:61 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34.

- 35 10. The bispecific antigen binding molecule of any one of claims 1 to 8, wherein the antigen binding domain capable of specific binding to OX40 comprises a heavy chain

variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:41 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:42.

11. The bispecific antigen binding molecule of any one of claims 1 to 10, wherein the
5 Fc region is an IgG, particularly an IgG1 Fc region or an IgG4 Fc region.

12. The bispecific antigen binding molecule of any one of claims 1 to 11, wherein the Fc region is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

13. The bispecific antigen binding molecule of any one of claims 1 to 12, wherein the
10 bispecific antigen binding molecule comprises

(a) at least two Fab fragments capable of specific binding to OX40 each connected to the N-terminus of one of subunits of the Fc region, and

(b) one cross-Fab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region, and

15 (c) the Fc region composed of a first and a second subunit capable of stable association.

14. The bispecific antigen binding molecule of claim 13, wherein the V_H-Ckappa chain of the cross-fab fragment capable of specific binding to FAP is fused to the C-terminus of one of subunits of the Fc region.

20 15. The bispecific antigen binding molecule of any one of claims 1 to 14, consisting of

(aa) a first Fab fragment capable of specific binding to OX40,

(ab) a second Fab fragment capable of specific binding to OX40,

25 (b) a cross-Fab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region, and

(c) the Fc region composed of a first and a second subunit capable of stable association, wherein the first Fab fragment (aa) is fused at the C-terminus of the V_H-CH1 chain to the N-terminus of the first subunit and the second Fab fragment (ab) is fused at the C-terminus of the V_H-CH1 chain to the N-terminus of the second subunit.

30 16. The bispecific antigen binding molecule of any one of claims 1 to 14, consisting of

(aa) a first Fab fragment capable of specific binding to OX40,

(ab) a second Fab fragment capable of specific binding to OX40,

- (ac) a third Fab fragment capable of specific binding to OX40,
 - (b) a cross-Fab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region, and
 - (c) the Fc region composed of a first and a second subunit capable of stable
- 5 association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the first subunit, and the third Fab fragment (ac) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit.
- 10 17. The bispecific antigen binding molecule of any one of claims 1 to 14, consisting of
- (aa) a first Fab fragment capable of specific binding to OX40,
 - (ab) a second Fab fragment capable of specific binding to OX40,
 - (ac) a third Fab fragment capable of specific binding to OX40,
 - 15 (ad) a fourth Fab fragment capable of specific binding to OX40,
 - (b) a cross-Fab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region, and
 - (c) the Fc region composed of a first and a second subunit capable of stable
- 20 association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the first subunit, and the fourth Fab fragment (ad) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the third Fab fragment (ac), which is in turn fused at its C-terminus to the N-terminus of the second subunit.
- 25 18. Isolated nucleic acid encoding the bispecific antigen binding molecule of any one of claims 1 to 17.
19. An expression vector comprising the isolated nucleic acid of claim 18.
20. A host cell comprising isolated nucleic acid of claim 18 or the expression vector of claim 19.
- 30 21. A method of producing a bispecific antigen binding molecule, comprising culturing the host cell of claim 20 under conditions suitable for the expression of the bispecific antigen binding molecule, and isolating the bispecific antigen binding molecule.
22. A pharmaceutical composition comprising the bispecific antigen binding molecule of any one of claims 1 to 17 and a pharmaceutically acceptable carrier.

23. The pharmaceutical composition of claim 22, further comprising an additional therapeutic agent.

24. The bispecific antigen binding molecule of any one of claims 1 to 17, or the pharmaceutical composition of claim 22, for use as a medicament.

5 25. The bispecific antigen binding molecule of any one of claims 1 to 17, or the pharmaceutical composition of claim 22, for use

(i) in inducing immune stimulation,

(ii) in stimulating tumor-specific T cell response,

(iii) in causing apoptosis of tumor cells,

10 (iv) in the treatment of cancer,

(v) in delaying progression of cancer,

(vi) in prolonging the survival of a patient suffering from cancer,

(vii) in the treatment of infections.

15 26. The bispecific antigen binding molecule of any one of claims 1 to 17, or the pharmaceutical composition of claim 22, for use in the treatment of cancer.

27. The bispecific antigen binding molecule according to any one of claims 1 to 17, or the pharmaceutical composition according to claim 22, for use in the treatment of cancer, wherein the bispecific antigen binding molecule or pharmaceutical composition is ~~for administration~~to be administered in combination with a chemotherapeutic agent,
20 radiation and/ or other agents for use in cancer immunotherapy.

28. The bispecific antigen binding molecule according to any one of claims 1 to 17, or the pharmaceutical composition according to claim 22, for use in the treatment of cancer, wherein the bispecific agonistic OX40 antigen binding molecule is ~~for administration~~to be administered in combination with a T-cell activating anti-CD3
25 bispecific antibody.

29. Use of the bispecific antigen binding molecule of any one of claims 1 to 17, or the pharmaceutical composition of claim 22, in the manufacture of a medicament for the treatment of cancer.

30 ~~30. A method of treating an individual having cancer comprising administering to the individual an effective amount of the bispecific antigen binding molecule of any one of claims 1 to 16, or the pharmaceutical composition of claim 21.~~