

The available pre-clinical and clinical data clearly demonstrate that there is a high clinical need for effective agonists of CD40 that are able to induce and enhance effective endogenous immune responses to cancer. However, almost never are the effects limited to a single type of cells or acting via a single mechanism and studies designed to elucidate inter- and intracellular signaling mechanisms have revealed increasing levels of complexity. Known CD40 antibodies can only be administered in relatively low doses due to dose-limiting toxicities such as cytokine release syndrome and thrombocyte/ endothelial cell activation, resulting in an insufficient activation of the pathway on target APCs and a narrow therapeutic index. Thus, there is a need of “targeted” agonists that preferably act on a single type of cells.

The invention relates to new bispecific antigen binding molecules capable of specific binding to CD40 and a target cell antigen. 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 CD40, wherein the activation of APCs through CD40 is provided by cross-linking through the target cell antigen, for example FAP expressed on tumor stroma cells and potentially also through FAP intermediately expressed in secondary lymphoid tissues. The FAP-dependent cross-linking of the bispecific antigen binding molecules confines the activation of CD40-expressing cells to the tumor tissue and potentially also to secondary lymphoid tissues such as tumor-draining lymph nodes. In contrast to bispecific antigen binding molecules capable of specific binding to CD40 and to immune checkpoint receptors on activated T cells, such as CTLA-4 or PD-1, targeting to a tumor target such as FAP enables CD40-mediated APC activation mainly in the tumor stroma and tumor-draining lymph nodes where fibroblasts express increased levels of FAP compared to other tissues. The antigen binding molecules of this invention may thus be able to trigger the CD40 receptor not only effectively, but also very selectively at the desired site while overcoming the need for FcγR cross-linking thereby reducing side effects.

SUMMARY

The present disclosure relates to bispecific antigen binding molecules combining at least one moiety (antigen binding domain) capable of specific binding to the costimulatory TNF receptor family member CD40, 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 CD40 receptors at the site where the target cell antigen is expressed, due to their binding capability towards a target cell antigen.

The disclosure provides a bispecific antigen binding molecule, comprising
(a) at least one antigen binding domain capable of specific binding to CD40, and
(b) at least one antigen binding domain capable of specific binding to a target cell antigen.

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

- 5 (a) at least one antigen binding domain capable of specific binding to CD40, and
(b) at least one antigen binding domain capable of specific binding to FAP comprising a heavy
chain variable region (VHFAP) comprising (i) CDR-H1 comprising the amino acid sequence of
SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, and (iii)
CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region
10 (VLFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:6, (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 connected to the C-terminus of the Fc region.

In a particular example, the bispecific antigen binding molecule comprises (a) at least one antigen binding domain capable of specific binding to CD40, (b) at least one antigen binding domain 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. More particularly, the Fc domain
5 composed of a first and a second subunit capable of stable association comprises mutations that reduce effector function.

In one example, the antigen binding domain capable of specific binding to CD40 binds to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:1.

In a further example, provided is a bispecific antigen binding molecule, wherein the
10 antigen binding domain capable of specific binding to a target cell antigen is an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP). In particular, the antigen binding domain capable of specific binding to FAP binds to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:2. Thus, in one example, the disclosure provides a bispecific antigen binding molecule, comprising (a) at least one antigen binding
15 domain capable of specific binding to CD40, and (b) at least one antigen binding domain capable of specific binding to FAP.

In one example, the disclosure provides a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to FAP comprises

(a) a heavy chain variable region (V_{HFAP}) comprising (i) CDR-H1 comprising the amino
20 acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_{LFAP}) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:6, (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, or

(b) a heavy chain variable region (V_{HFAP}) comprising (i) CDR-H1 comprising the amino acid
25 sequence of SEQ ID NO:11, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:13, and a light chain variable region (V_{LFAP}) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:15, and (vi)
30 CDR-L3 comprising the amino acid sequence of SEQ ID NO:16.

In a further example, provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to FAP comprises

(a) a heavy chain variable region (V_{HFAP}) 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
35 NO:9, and a light chain variable region (V_{LFAP}) 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:10, or

(b) a heavy chain variable region (V_HFAP) 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

- 5 NO:17, and a light chain variable region (V_LFAP) 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:18.

In particular, provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to FAP comprises (a) a heavy

- 10 chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:9, and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:10, or

(b) a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:18.

- 15 In a further example, provided is a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to CD40 comprises a heavy chain variable region (V_HCD40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:19, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21, and a light chain variable region
- 20 (V_LCD40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:22, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:23, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:24.

In another example, provided is a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to CD40 binds to mouse CD40 and comprises a

- 25 heavy chain variable region (V_HCD40) 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_LCD40) 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)
- 30 CDR-L3 comprising the amino acid sequence of SEQ ID NO:32.

Furthermore, provided is a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to CD40 comprises (a) a V_H comprising the amino acid sequence of SEQ ID NO:25 and a V_L comprising the amino acid sequence of SEQ ID NO:26, or (b) a V_H comprising the amino acid sequence of SEQ ID NO:33 and a V_L comprising

35 the amino acid sequence of SEQ ID NO:34. In a particular example, the disclosure provides a

bispecific antigen binding molecule, wherein each of the antigen binding domains capable of specific binding to CD40 comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a VL comprising the amino acid sequence of SEQ ID NO:26.

In a further example, provided is a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to CD40 comprises a heavy chain variable region (V_HCD40) comprising

(i) CDR-H1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:35,

(ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:20, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43 and SEQ ID NO:44, and

(iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21, and a light chain variable region (V_LCD40) comprising

(iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:22,

(v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:23, and

(vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:24.

In yet another example, provided is a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to CD40 comprises a heavy chain variable region (V_HCD40) comprising

(i) CDR-H1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:261,

(ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:20, SEQ ID NO:262 and SEQ ID NO:263, and

(iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21,

and a light chain variable region (V_LCD40) comprising

(iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:22, SEQ ID NO:264 and SEQ ID NO:265,

(v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:23, and

(vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:24.

Furthermore, provided is a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to CD40 comprises

(i) a heavy chain variable region (V_HCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 and SEQ ID NO:174, and

More particularly, provided is a bispecific antigen binding, wherein the antigen binding domain capable of specific binding to CD40 comprises a VH comprising the amino acid sequence of SEQ ID NO:171 and a VL comprising the amino acid sequence of SEQ ID NO:175.

In a further example, provided is a bispecific antigen binding molecule of the disclosure,
5 wherein the antigen binding domain capable of specific binding to CD40 comprises

(i) a heavy chain variable region (V_HCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183 and SEQ ID NO:184, and

(ii) a light chain variable region (V_LCD40) comprising the amino acid sequence selected
10 from the group consisting of SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, and SEQ ID NO:188.

In particular, a bispecific antigen binding molecule is provided, wherein the antigen binding domain capable of specific binding to CD40 comprises

(a) a VH comprising the amino acid sequence of SEQ ID NO:179 and a VL comprising the
15 amino acid sequence of SEQ ID NO:185, or

(b) a VH comprising the amino acid sequence of SEQ ID NO:180 and a VL comprising the amino acid sequence of SEQ ID NO:185, or

(c) a VH comprising the amino acid sequence of SEQ ID NO:181 and a VL comprising the amino acid sequence of SEQ ID NO:185, or

(d) a VH comprising the amino acid sequence of SEQ ID NO:182 and a VL comprising the
20 amino acid sequence of SEQ ID NO:185, or

(e) a VH comprising the amino acid sequence of SEQ ID NO:179 and a VL comprising the amino acid sequence of SEQ ID NO:186, or

(f) a VH comprising the amino acid sequence of SEQ ID NO:180 and a VL comprising the
25 amino acid sequence of SEQ ID NO:186, or

(g) a VH comprising the amino acid sequence of SEQ ID NO:181 and a VL comprising the amino acid sequence of SEQ ID NO:186, or

(h) a VH comprising the amino acid sequence of SEQ ID NO:182 and a VL comprising the amino acid sequence of SEQ ID NO:186, or

(i) a VH comprising the amino acid sequence of SEQ ID NO:183 and a VL comprising the
30 amino acid sequence of SEQ ID NO:187, or

(j) a VH comprising the amino acid sequence of SEQ ID NO:183 and a VL comprising the amino acid sequence of SEQ ID NO:188, or

(k) a VH comprising the amino acid sequence of SEQ ID NO:184 and a VL comprising the
35 amino acid sequence of SEQ ID NO:187, or

(l) a VH comprising the amino acid sequence of SEQ ID NO:184 and a VL comprising the amino acid sequence of SEQ ID NO:188.

More particularly, provided is a bispecific antigen binding, wherein the antigen binding domain capable of specific binding to CD40 comprises a VH comprising the amino acid sequence of SEQ ID NO:179 and a VL comprising the amino acid sequence of SEQ ID NO:185 or wherein the antigen binding domain capable of specific binding to CD40 comprises a VH
5 comprising the amino acid sequence of SEQ ID NO:182 and a VL comprising the amino acid sequence of SEQ ID NO:185.

In another example, provided is a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to CD40 comprises

(i) a heavy chain variable region (V_HCD40) comprising an amino acid sequence selected
10 from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54 and SEQ ID NO:55, and

(ii) a light chain variable region (V_LCD40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59,
15 SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63 and SEQ ID NO:64.

Particularly, a bispecific antigen binding molecule is provided, wherein each of the moieties capable of specific binding to CD40 comprises a VH comprising the amino acid sequence of SEQ ID NO:47 and a VL comprising the amino acid sequence of SEQ ID NO:57.

Furthermore, provided is a bispecific antigen binding molecule comprising

(i) at least one antigen binding domain capable of specific binding to CD40, comprising a
20 heavy chain variable region (V_HCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183 and SEQ ID NO:184, and a light chain variable region (V_LCD40) comprising an amino acid sequence
25 selected from the group consisting of SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187 and SEQ ID NO:188, and

(ii) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:9 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:10, or a
30 heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:17 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:18.

In another example, provided is a bispecific antigen binding molecule comprising

(i) at least one antigen binding domain capable of specific binding to CD40, comprising a heavy chain variable region (V_HCD40) comprising an amino acid sequence selected from the
35 group consisting of SEQ ID NO:25, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID

NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54 and SEQ ID NO:55, and a light chain variable region (V_LCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63 and SEQ ID NO:64, and

(ii) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:9 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:10, or a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:17 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:18.

In one example, the bispecific antigen binding molecule is a humanized or a chimeric antibody. In a further example, 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 a particular example, provided is a bispecific antigen binding molecule, wherein the Fc region is (i) of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index), or (ii) of mouse IgG1 subclass with the amino acid mutations D265A and P329G (numbering according to Kabat EU index). Particularly, 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 example, 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 (i) 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), or (ii) 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 example, 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 CD40 connected to a Fc region, and

(b) at least one antigen binding domain capable of specific binding to FAP connected to the C-terminus of the Fc region.

5 In another example, 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 CD40 connected to a Fc region, and

10 (b) one antigen binding domain capable of specific binding to FAP connected to the C-terminus of the Fc region.

In a particular example, the antigen binding domain capable of specific binding to FAP connected to the C-terminus of the Fc region is a cross-fab fragment. Thus, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule comprises

15 (a) at least two Fab fragments capable of specific binding to CD40 connected to a Fc region, and

(b) a cross-fab fragment capable of specific binding to FAP connected to the C-terminus of the Fc region.

In one example, provided is a bispecific antigen binding molecule comprising

20 (a) two light chains and two heavy chains of an antibody comprising two Fab fragments capable of specific binding to CD40, and a Fc region, and

(b) a VH and a VL of an antigen binding domain capable specific binding to FAP, wherein the VH is connected to the C-terminus of one of the two heavy chains of (a), and wherein the VL is connected to the C-terminus of the other of the two heavy chains of (a).

In another example, provided is a bispecific antigen binding molecule comprising

25 (a) two light chains and two heavy chains of an antibody comprising two Fab fragments capable of specific binding to CD40, and a Fc region, and

(b) a cross-fab fragment capable specific binding to FAP, wherein the VH-CL chain is connected to the C-terminus of one of the two heavy chains of (a).

In yet another example, provided is a bispecific antigen binding molecule comprising

30 (a) two light chains and two heavy chains of an antibody comprising two Fab fragments capable of specific binding to CD40, and a Fc region, and

(b) a cross-fab fragment capable specific binding to FAP, wherein the VL-CH1 chain is connected to the C-terminus of one of the two heavy chains of (a).

Furthermore, provided is a bispecific antigen binding molecule comprising

(a) two light chains and two heavy chains of an antibody comprising two Fab fragments capable of specific binding to CD40, and a Fc region, and

(b) two Fab fragments capable of specific binding to FAP, wherein one of the Fab fragments is connected to the C-terminus of one of the two heavy chains of (a), and the other of
5 the Fab fragments is connected to the C-terminus of the other of the two heavy chains of (a).

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

(a) two heavy chains, each heavy chain comprising a VH and CH1 domain of a Fab fragment capable of specific binding to CD40 and a Fc region subunit,

10 (b) two light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to CD40, and

(c) a VH and a VL of an antigen binding domain capable of specific binding to FAP, wherein the VH is connected to the C-terminus of one of the two heavy chains of (a), and wherein the VL is connected to the C-terminus of the other of the two heavy chains of (a).

15 In a further example, provided is a bispecific antigen binding molecule comprising

(a) two heavy chains, each heavy chain comprising a VH and CH1 domain of a Fab fragment capable of specific binding to CD40, and a Fc region subunit,

(b) two light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to CD40, and

20 (c) two Fab fragments capable of specific binding to FAP, wherein one of the Fab fragments is connected to the C-terminus of one of the two heavy chains of (a), and the other of the Fab fragments is connected to the C-terminus of the other of the two heavy chains of (a).

In another example, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule comprises

25 (a) two heavy chains, each heavy chain comprising a VH and CH1 domain of a Fab fragment capable of specific binding to CD40, and a Fc region subunit,

(b) two light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to CD40, and

30 (c) one Fab fragment capable of specific binding to FAP, wherein the Fab fragments is connected to the C-terminus of one of the two heavy chains of (a).

In another example, the Fab fragment or the two Fab fragments capable of specific binding to FAP are crossover Fab fragments each comprising a VL-CH1 chain and a VH-CL chain, and wherein the VH-CL chain or the VL-CH1 chain is connected to the C-terminus of one of the two heavy chains of (a).

35 In one example, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule comprises four Fab fragments capable of specific binding to CD40. In

a particular example, provided is a bispecific antigen binding molecule, wherein each of the two heavy chains of (a) as defined herein before comprises two VH-CH1 chains of a Fab fragment capable of specific binding to CD40 that are connected to each other, optionally by a peptide linker.

5 In another example, the 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 CD40 that are connected to each other, optionally by a peptide linker, and a Fc region subunit,

10 (b) four light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to CD40, and

 (c) a VH and a VL of an antigen binding domain capable of specific binding to FAP, wherein the VH is connected to the C-terminus of one of the two heavy chains of (a), and wherein the VL is connected to the C-terminus of the other of the two heavy chains of (a).

15 In another example, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule comprises

 (a) two heavy chains, each heavy chain comprising two VH-CH1 chains of a Fab fragment capable of specific binding to CD40 that are connected to each other, optionally by a peptide linker, and a Fc region subunit,

20 (b) four light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to CD40, and

 (c) one Fab fragment or cross-Fab fragment capable of specific binding to FAP, wherein the Fab or cross-Fab fragment is connected to the C-terminus of one of the two heavy chains of (a).

25 In another example, provided is 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 CD40 that are connected to each other, optionally by a peptide linker, and a Fc region subunit,

30 (b) four light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to CD40, and

 (c) a cross-fab fragment capable specific binding to FAP, wherein the VH-CL chain of said cross-fab fragment is connected to the C-terminus of one of the two heavy chains of (a).

 In yet another example, provided is a bispecific antigen binding molecule comprising

35 (a) two heavy chains, each heavy chain comprising two VH-CH1 chains of a Fab fragment capable of specific binding to CD40 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 CL domain of a Fab fragment capable of specific binding to CD40, and

(c) a cross-fab fragment capable specific binding to FAP, wherein the VL-CH1 chain of said cross-fab fragment is connected to the C-terminus of one of the two heavy chains of (a).

5 In another particular example, provided is a bispecific antigen binding molecule, wherein one or more of the Fab fragments capable of specific binding to CD40 comprises a CL domain comprising an arginine (R) at amino acid at position 123 (numbering according to Kabat EU index) and a lysine (K) at amino acid at position 124 (numbering according to Kabat EU index),
10 and a CH1 domain comprising a glutamic acid (E) at amino acid at position 147 (numbering according to Kabat EU index) and a glutamic acid (E) at amino acid at position 213 (numbering according to Kabat EU index).

 According to the disclosure, there is provided an isolated polynucleotide encoding a bispecific antigen binding molecule as described herein before. In another aspect, provided is a polynucleotide encoding the bispecific antigen binding molecule of the disclosure. The
15 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 expression vector of the disclosure. In another aspect, provided is an expression vector comprising the polynucleotide of the disclosure. In another aspect, provided is a host cell comprising polynucleotide of the disclosure or the expression vector of the disclosure. In some
20 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 disclosure also encompasses the bispecific antigen
25 binding molecule that specifically binds to CD40 and to FAP produced by the method of the disclosure.

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

Also encompassed by the disclosure is the bispecific antigen binding molecule as described herein before, or the pharmaceutical composition comprising the bispecific antigen binding molecule, for use as a medicament. In another aspect, provided is the bispecific antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use as a
5 medicament.

Provided is a bispecific antigen binding molecule as described herein before or the pharmaceutical composition of the disclosure, for use

(i) in inducing immune stimulation by CD40 expressing antigen-presenting cells (APCs),

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

10 (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,
- (vii) in the treatment of infections.

5 In another aspect, provided is the bispecific antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment of infections.

10 In a specific aspect, provided is the bispecific antigen binding molecule as described herein before or the pharmaceutical composition of the disclosure, for use in the treatment of cancer. The disclosure provides the bispecific antigen binding molecule as described herein before for use in the treatment of cancer, wherein the bispecific antigen binding molecule is administered in combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy. In another aspect, the disclosure provides the bispecific antigen binding molecule of the disclosure or the pharmaceutical composition of the disclosure for use in combination cancer therapy, 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. Provided is the bispecific antigen binding molecule as described herein before or the pharmaceutical composition of the disclosure, for use in up-regulating or prolonging cytotoxic T cell activity.

20 The disclosure provides a method of inhibiting the growth of tumor cells in an individual comprising administering to the individual an effective amount of the bispecific antigen binding molecule as described herein before, or the pharmaceutical composition of the disclosure, to inhibit the growth of the tumor cells. The disclosure provides a method of treating or delaying cancer in an individual comprising administering to the individual an effective amount of the bispecific antigen binding molecule as described herein before, or the pharmaceutical composition of the disclosure.

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

5 individual a therapeutically effective amount of a composition comprising the bispecific antigen binding molecule of the disclosure in a pharmaceutically acceptable form. In a specific example, the disease is cancer. In any of the above examples the individual is a mammal, particularly a human.

BRIEF DESCRIPTION OF THE DRAWINGS

10 **Figures 1A to 1F** show schematic representations of the bispecific antigen binding molecules which specifically bind to human CD40 and to FAP. **Fig. 1A** shows a schematic representation of a bispecific CD40-FAP antibody in the 4+1 format consisting of four CD40 binding Fab domains combined with one FAP binding moiety with VH at the C-terminus of one heavy chain and VL at the C-terminus of the other heavy chain (tetravalent for CD40 and

15 monovalent for FAP). The black point symbolizes knob-into-hole mutations. **Fig. 1B** shows a schematic representation of a bispecific CD40-FAP antibody in the 4+2 format consisting of four CD40 binding Fab domains combined with two FAP binding Fab domains fused each at the C-

Claims

1. A bispecific antigen binding molecule, comprising
 - (a) at least one antigen binding domain capable of specific binding to CD40, and
 - 5 (b) at least one antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V_{HFAP}) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:4, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_{LFAP}) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:6, (v)
 - 10 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 connected to the C-terminus of the Fc region.
2. The bispecific antigen binding molecule of claim 1, additionally comprising
 - (c) a Fc region composed of a first and a second subunit capable of stable association.
3. The bispecific antigen binding molecule of claim 1 or claim 2, wherein the antigen
- 15 binding domain capable of specific binding to CD40 binds to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:1.
4. The bispecific antigen binding molecule of any one of claims 1 to 3, wherein the antigen binding domain capable of specific binding to FAP comprises
 - (a) a heavy chain variable region (V_{HFAP}) comprising an amino acid sequence that is at least
 - 20 about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:9, and a light chain variable region (V_{LFAP}) 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:10, or
 - (b) a heavy chain variable region (V_{HFAP}) comprising an amino acid sequence that is at least
 - 25 about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_{LFAP}) 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:18.
5. The bispecific antigen binding molecule of any one of claims 1 to 4, wherein the antigen
- 30 binding domain capable of specific binding to CD40 comprises a heavy chain variable region (V_{HCD40}) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:19, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21, and a light chain variable region (V_{LCD40}) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:22, (v) CDR-L2

comprising the amino acid sequence of SEQ ID NO:23, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:24.

6. The bispecific antigen binding molecule of any one of claims 1 to 4, wherein the antigen binding domain capable of specific binding to CD40 comprises a heavy chain variable region (V_HCD40) 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_LCD40) 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.

7. The bispecific antigen binding molecule of any one of claims 1 to 6, wherein the antigen binding domain capable of specific binding to CD40 comprises

(a) a V_H comprising the amino acid sequence of SEQ ID NO:25 and a V_L comprising the amino acid sequence of SEQ ID NO:26, or

(b) a V_H comprising the amino acid sequence of SEQ ID NO:33 and a V_L comprising the amino acid sequence of SEQ ID NO:34.

8. The bispecific antigen binding molecule of any one of claims 1 to 5, wherein the antigen binding domain capable of specific binding to CD40 comprises

(i) a heavy chain variable region (V_HCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 and SEQ ID NO:174, and

(ii) a light chain variable region (V_LCD40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, and SEQ ID NO:178.

9. The bispecific antigen binding molecule of any one of claims 1 to 5, wherein the antigen binding domain capable of specific binding to CD40 comprises

(i) a heavy chain variable region (V_HCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183 and SEQ ID NO:184, and

(ii) a light chain variable region (V_LCD40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, and SEQ ID NO:188.

10. The bispecific antigen binding molecule of any one of claims 1 to 5 or 8, wherein the antigen binding domain capable of specific binding to CD40 comprises

(a) a VH comprising the amino acid sequence of SEQ ID NO:171 and a VL comprising the amino acid sequence of SEQ ID NO:175, or

(b) a VH comprising the amino acid sequence of SEQ ID NO:173 and a VL comprising the amino acid sequence of SEQ ID NO:177, or

5 (c) a VH comprising the amino acid sequence of SEQ ID NO:174 and a VL comprising the amino acid sequence of SEQ ID NO:178, or

(d) a VH comprising the amino acid sequence of SEQ ID NO:171 and a VL comprising the amino acid sequence of SEQ ID NO:177, or

10 (e) a VH comprising the amino acid sequence of SEQ ID NO:171 and a VL comprising the amino acid sequence of SEQ ID NO:178, or

(f) a VH comprising the amino acid sequence of SEQ ID NO:173 and a VL comprising the amino acid sequence of SEQ ID NO:175, or

(g) a VH comprising the amino acid sequence of SEQ ID NO:173 and a VL comprising the amino acid sequence of SEQ ID NO:178, or

15 (h) a VH comprising the amino acid sequence of SEQ ID NO:174 and a VL comprising the amino acid sequence of SEQ ID NO:175, or

(i) a VH comprising the amino acid sequence of SEQ ID NO:174 and a VL comprising the amino acid sequence of SEQ ID NO:177, or

20 (j) a VH comprising the amino acid sequence of SEQ ID NO:171 and a VL comprising the amino acid sequence of SEQ ID NO:176, or

(k) a VH comprising the amino acid sequence of SEQ ID NO:172 and a VL comprising the amino acid sequence of SEQ ID NO:175, or

(l) a VH comprising the amino acid sequence of SEQ ID NO:172 and a VL comprising the amino acid sequence of SEQ ID NO:176, or

25 (m) a VH comprising the amino acid sequence of SEQ ID NO:172 and a VL comprising the amino acid sequence of SEQ ID NO:177, or

(n) a VH comprising the amino acid sequence of SEQ ID NO:172 and a VL comprising the amino acid sequence of SEQ ID NO:178, or

30 (o) a VH comprising the amino acid sequence of SEQ ID NO:173 and a VL comprising the amino acid sequence of SEQ ID NO:176, or

(p) a VH comprising the amino acid sequence of SEQ ID NO:174 and a VL comprising the amino acid sequence of SEQ ID NO:176.

11. The bispecific antigen binding molecule of any one of claims 1 to 5 or 8 or 10, wherein the antigen binding domain capable of specific binding to CD40 comprises a VH comprising the amino acid sequence of SEQ ID NO:171 and a VL comprising the amino acid sequence of SEQ ID NO:175.

- 5 12. The bispecific antigen binding molecule of any one of claims 1 to 5 or 9, wherein the antigen binding domain capable of specific binding to CD40 comprises
- (a) a VH comprising the amino acid sequence of SEQ ID NO:179 and a VL comprising the amino acid sequence of SEQ ID NO:185, or
- (b) a VH comprising the amino acid sequence of SEQ ID NO:180 and a VL comprising the amino acid sequence of SEQ ID NO:185, or
- 10 (c) a VH comprising the amino acid sequence of SEQ ID NO:181 and a VL comprising the amino acid sequence of SEQ ID NO:185, or
- (d) a VH comprising the amino acid sequence of SEQ ID NO:182 and a VL comprising the amino acid sequence of SEQ ID NO:185, or
- 15 (e) a VH comprising the amino acid sequence of SEQ ID NO:179 and a VL comprising the amino acid sequence of SEQ ID NO:186, or
- (f) a VH comprising the amino acid sequence of SEQ ID NO:180 and a VL comprising the amino acid sequence of SEQ ID NO:186, or
- (g) a VH comprising the amino acid sequence of SEQ ID NO:181 and a VL comprising the amino acid sequence of SEQ ID NO:186, or
- 20 (h) a VH comprising the amino acid sequence of SEQ ID NO:182 and a VL comprising the amino acid sequence of SEQ ID NO:186, or
- (i) a VH comprising the amino acid sequence of SEQ ID NO:183 and a VL comprising the amino acid sequence of SEQ ID NO:187, or
- 25 (j) a VH comprising the amino acid sequence of SEQ ID NO:183 and a VL comprising the amino acid sequence of SEQ ID NO:188, or
- (k) a VH comprising the amino acid sequence of SEQ ID NO:184 and a VL comprising the amino acid sequence of SEQ ID NO:187, or
- (l) a VH comprising the amino acid sequence of SEQ ID NO:184 and a VL comprising the amino acid sequence of SEQ ID NO:188.
- 30

13. The bispecific antigen binding molecule of any one of claims 1 to 5 or 9 or 12, wherein the antigen binding domain capable of specific binding to CD40 comprises a VH comprising the

amino acid sequence of SEQ ID NO:179 and a VL comprising the amino acid sequence of SEQ ID NO:185 or wherein the antigen binding domain capable of specific binding to CD40 comprises a VH comprising the amino acid sequence of SEQ ID NO:182 and a VL comprising the amino acid sequence of SEQ ID NO:185.

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

 (i) at least one antigen binding domain capable of specific binding to CD40, comprising a heavy chain variable region (V_HCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183 and SEQ ID
10 NO:184, and a light chain variable region (V_LCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187 and SEQ ID NO:188, and

 (ii) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:9 and a
15 light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:10, or a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:17 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:18.

 15. The bispecific antigen binding molecule of any one of claims 2 to 14, wherein the Fc region is an IgG, particularly an IgG1 Fc region or an IgG4 Fc region and wherein the Fc region
20 comprises one or more amino acid substitution that reduces the binding affinity of the antibody to an Fc receptor and/or effector function.

 16. The bispecific antigen binding molecule of any one of claims 2 to 15, wherein the Fc region is (i) of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index), or (ii) of mouse IgG1 subclass with the amino acid
25 mutations D265A and P329G (numbering according to Kabat EU index).

 17. The bispecific antigen binding molecule of any one of claims 1 to 16, wherein the bispecific antigen binding molecule comprises

 (a) at least two Fab fragments capable of specific binding to CD40 connected to a Fc region, and

30 (b) one antigen binding domain capable of specific binding to FAP connected to the C-terminus of the Fc region.

 18. The bispecific antigen binding molecule of any one of claims 1 to 17, wherein the bispecific antigen binding molecule comprises

(a) at least two Fab fragments capable of specific binding to CD40 connected to a Fc region, and

(b) a cross-fab fragment capable of specific binding to FAP connected to the C-terminus of the Fc region.

5 19. The bispecific antigen binding molecule of any one of claims 1 to 17, wherein the bispecific antigen binding molecule comprises four Fab fragments capable of specific binding to CD40.

20. Polynucleotide encoding the bispecific antigen binding molecule of any one of claims 1 to 19.

10 21. An expression vector comprising the polynucleotide of claim 20.

22. A host cell comprising polynucleotide of claim 18 or the expression vector of claim 21.

23. A method of producing a bispecific antigen binding molecule, comprising culturing the host cell of claim 22 under conditions suitable for the expression of the bispecific antigen binding molecule, and isolating the bispecific antigen binding molecule.

15 24. A pharmaceutical composition comprising the bispecific antigen binding molecule of any one of claims 1 to 19 and at least one pharmaceutically acceptable excipient.

25. The bispecific antigen binding molecule of any one of claims 1 to 19, or the pharmaceutical composition of claim 24, for use as a medicament.

20 26. The bispecific antigen binding molecule of any one of claims 1 to 19, or the pharmaceutical composition of claim 24, for use in the treatment of infections.

27. The bispecific antigen binding molecule of any one of claims 1 to 19, or the pharmaceutical composition of claim 24, for use in the treatment of cancer.

25 28. The bispecific antigen binding molecule according to any one of claims 1 to 19 or the pharmaceutical composition according to claim 24 for use in combination cancer therapy, 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.
