

Bispecific 2+1 Contorsbodies

FIELD OF THE INVENTION

The invention relates to novel bispecific antibodies (contorsbodies) consisting of two fusion polypeptides comprising two antigen binding domains capable of specific binding to a first target and one antigen binding domain capable of specific binding to a second target, and to
5 methods of producing these molecules and to methods of using the same.

BACKGROUND

Since the development of the first monoclonal antibodies by Koehler and Milstein in 1974 a lot of efforts have been dedicated to the development of antibodies which are appropriate for therapy in humans. The first monoclonal antibodies which became available had been developed
10 in mice and rats. These antibodies when used for therapy of a human being caused unwanted side effects due to anti-rodent antibodies. A lot of efforts have been dedicated to the reduction or even elimination of such unwanted side effects. In the past years an ever growing number of human monoclonal antibodies or humanized monoclonal antibodies have reached the market.

Bispecific antibodies have become of increasing interest for diagnostic and therapeutic
15 applications. While natural antibodies are monospecific, bispecific antibodies recognize two different epitopes either on the same or on different antigens. Over the past years, a plethora of new antibody formats have been developed. The application of sophisticated molecular design and genetic engineering has solved many of the technical problems associated with the formation of bispecific antibodies such as stability, solubility and other parameters that confer drug
20 properties and that are summarized under the term “developability”. In addition, different desired features of the bispecific antibody to be generated (i.e. target product profiles) make it necessary to have access to a diverse panel of antibody formats. These formats may vary in size, geometry of their binding modules, valencies, flexibility as well as in their pharmacokinetic properties (Brinkmann U. and Kontermann R.E., MABS 2017, 9(2), 182-212).

25 However, it seems that there is not one best format for all needs and that there is still potential to optimize antibody formats derived from the wild-type four chain Y-shaped antibody format. For the use as pharmaceutical product, bispecific antibodies need to be produced in large amounts in a reproducible manner, preferably at high yields. The more complex composition (e.g. 3-4 chains in contrast to 2-chain IgGs) does often require more extensive optimization of

expression systems. Furthermore, the presence or absence of undesired side products can be of high importance.

SUMMARY OF THE INVENTION

The present invention refers to bispecific antibodies that particularly consist of two chains
5 although they comprise three antigen binding domains. The antibodies of the present invention differ in the spatial orientation of the antigen binding domain from classical antibodies in the IgG format.

The present invention provides a bispecific antibody consisting of two fusion polypeptides and comprising two antigen binding domains capable of specific binding to a first target and one
10 antigen binding domain capable of specific binding to a second target, wherein

(a) the first fusion polypeptide comprises a first part of a first antigen binding domain capable of specific binding to the first target, a spacer domain, a second part of a first antigen binding domain capable of specific binding to the first target and a first part of an antigen binding domain capable of specific binding to the second target,
15 wherein

- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
- the first part of the first antigen binding domain capable of specific binding to the first target is fused either directly or via a first peptide linker to the N-terminus of the spacer domain,
- 20 - the second part of the first antigen binding domain capable of specific binding to the first target is fused either directly or via a second peptide linker to the C-terminus of the spacer domain, and
- the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the
25 second part of the first antigen binding domain capable of specific binding to the first target or is fused either directly or via a third peptide linker to the N-terminus of the first part of the first antigen binding domain capable of specific binding to the first target, and

(b) the second fusion polypeptide comprising a first part of a second antigen binding
30 domain capable of specific binding to a first target, a spacer domain, a second part of the second antigen binding domain capable of specific binding to a first target and the second part of an antigen binding domain capable of specific binding to a second target, wherein

- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,

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- the first part of the second antigen binding domain capable of specific binding to a first target is fused either directly or via a first peptide linker to the N-terminus of the spacer domain,

5 - the second part of the second antigen binding domain capable of specific binding to a first target is fused either directly or via a second peptide linker to the C-terminus of the spacer domain, and

10 - the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the second antigen binding domain capable of specific binding to a first target or is fused either directly or via a third peptide linker to the N-terminus of the first part of the second antigen binding domain capable of specific binding to a first target,

15 wherein the first part and the second part of the antigen binding domain capable of specific binding to the second target are associated with each other to form the antigen binding domain capable of specific binding to the second target and wherein the first part and the second part of the first and second antigen binding domain capable of specific binding to the first target are associated with each other to form a circular fusion polypeptide, and
20 wherein the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide are associated covalently to each other by a disulfide bond and comprise modifications promoting the association of the first and second fusion polypeptide.

25 In one aspect, provided is a bispecific antibody as defined herein before, wherein in the first fusion polypeptide the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the first antigen binding domain capable of specific binding to the first target and wherein in the second fusion polypeptide the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the first antigen binding domain capable of specific binding to the first target.

30 In another aspect, provided is a bispecific antibody as defined herein before, wherein in the first fusion polypeptide the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the N-terminus of the first part of the first antigen binding domain capable of specific binding to the first target and wherein in the second fusion polypeptide the second part of an antigen binding domain capable of
35 specific binding to a second target is fused either directly or via a third peptide linker to the N-

terminus of the first part of the first antigen binding domain capable of specific binding to the first target.

In one aspect, provided is a bispecific antibody as defined herein before, wherein the third peptide linker connecting the first part or the second part of an antigen binding domain capable of specific binding to a second target comprises at least 15 amino acids. In one aspect, the third peptide linker connecting the first part of an antigen binding domain capable of specific binding to a second target and the third peptide linker connecting the second part of an antigen binding domain capable of specific binding to a second target are identical. In one aspect, the third peptide linker comprises 15 to 25 amino acids. In one particular aspect, the third peptide linker comprises the amino acid sequence of SEQ ID NO:84.

In one aspect, the invention provides a bispecific antibody as defined herein before, wherein the first fusion polypeptide comprises the heavy chain variable domain of the antigen binding domain capable of specific binding to a second target and the second fusion polypeptide comprises the antibody light chain variable domain of the antigen binding domain capable of specific binding to a second target or vice versa. In one particular aspect, the first part of the antigen binding domain is an antibody heavy chain Fab fragment and the second part of the antigen binding domain is an antibody light chain Fab fragment or vice versa. In one aspect, the first part of the antigen binding domain and the second part of the antigen binding domain are associated covalently to each other by a disulfide bond.

Thus, in one aspect, the first part of the antigen binding domain is an antibody heavy chain Fab fragment (VH-CH1) and the second part of the antigen binding domain is an antibody light chain Fab fragment (VL-Ckappa). In another aspect, the first part of the antigen binding domain is an antibody light chain Fab fragment and the second part of the antigen binding domain is an antibody heavy chain Fab fragment. In another aspect, the first part of the antigen binding domain is an antibody cross Fab fragment comprising VH-Ckappa and the second part of the antigen binding domain is an antibody cross Fab fragment comprising VL-CH1. In a further aspect, the first part of the antigen binding domain is an antibody cross Fab fragment comprising VL-CH1 and the second part of the antigen binding domain is an antibody cross Fab fragment comprising VH-Ckappa.

As described above, the bispecific antibody consists of a first and a second fusion polypeptide, both comprising a spacer domain, the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide are associated covalently to each other by a disulfide bond and comprise modifications promoting the association of the first and second fusion polypeptide. The spacer domain comprises at least 25 amino acids.

In one aspect of the invention, the spacer domain comprises an antibody hinge region or a (C-terminal) fragment thereof and an antibody CH2 domain or a (N-terminal) fragment thereof. In another aspect, the spacer domain comprises an antibody hinge region or a fragment thereof, an antibody CH2 domain, and an antibody CH3 domain or a fragment thereof. Furthermore, the
5 spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide comprise modifications promoting the association of the first and second fusion polypeptide. In a particular aspect, the spacer domain of the first fusion polypeptide comprises holes and the spacer domain of the second fusion polypeptide comprises knobs according to the knobs into hole method. In a further aspect, the invention comprises a bispecific antibody,
10 wherein the spacer domain comprises an antibody hinge region or a fragment thereof and an IgG1 Fc domain. Particularly, the IgG1 Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcγ receptor. More particularly, the IgG1 Fc domain comprises the amino acid substitutions L234A, L235A and P329G (numbering according to Kabat EU index).

15 In some aspects, provided is a bispecific antibody wherein the one antigen binding domain capable of specific binding to a second target is an antigen binding domain capable of specific binding to a tumor associated antigen (TAA). In particular, the tumor associated antigen is Fibroblast Activation Protein (FAP). In one aspect, provided is a bispecific antibody, wherein the antigen binding domain capable of specific binding to a second target is an antigen binding
20 domain capable of specific binding to Fibroblast Activation Protein (FAP).

In some aspects, the antigen binding domain capable of specific binding to FAP comprises (a) a heavy chain variable region (VHFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:3, and a light chain
25 variable region (VLFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:4, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:5, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:6, or
(b) a heavy chain variable region (VHFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain
30 variable region (VLFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:12, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:13, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:14.

More particularly, the antigen binding domain capable of specific binding to FAP
35 comprises
(a) a heavy chain variable region (VHFAP) 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:7, and a light chain variable region (VLFAP) 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:8, or

- 5 (b) a heavy chain variable region (VHFAP) 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:15, and a light chain variable region (VLFAP) 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:16.

- 10 In some aspects, provided is a bispecific antibody wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to a TNF receptor, in particular a costimulatory TNF receptor. Particularly, the costimulatory TNF receptor is OX40. In one aspect, provided is a bispecific antibody, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding
15 domain capable of specific binding to OX40. Particularly, the bispecific antibody of the invention comprises two antigen binding domains capable of specific binding to OX40.

In some aspects, the antigen binding domain capable of specific binding to OX40 comprises

- (a) a heavy chain variable region (VHOX40) comprising (i) CDR-H1 comprising the amino acid
20 sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a light chain variable region (VLOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:35, or
25 (b) a heavy chain variable region (VHOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21, and a light chain variable region (VLOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:34, or
(c) a heavy chain variable region (VHOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:23, and a light chain variable region (VLOX40) comprising (iv) CDR-L1 comprising the amino acid sequence
35 of SEQ ID NO:28, (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:36, or
(d) a heavy chain variable region (VHOX40) comprising (i) CDR-H1 comprising the amino acid

- sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:24, and a light chain variable region (VLOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi)
- 5 CDR-L3 comprising the amino acid sequence of SEQ ID NO:37, or
- (e) a heavy chain variable region (VHOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (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:25, and a light chain variable region (VLOX40) comprising (iv) CDR-L1 comprising the amino acid sequence
- 10 of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or
- (f) a heavy chain variable region (VHOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (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:26, and a light
- 15 chain variable region (VLOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or
- (g) a heavy chain variable region (VHOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID
- 20 NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:27, and a light chain variable region (VLOX40) 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:33, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:39.

- In particular, the antigen binding domain capable of specific binding to OX40 comprises a
- 25 heavy chain variable region (VHOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a light chain variable region (VLOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:35.

- In some aspects, the antigen binding domain capable of specific binding to OX40 comprises
- (a) a heavy chain variable region (VHOX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (VLOX40) comprising an amino acid sequence of SEQ
- 35 ID NO:41, or
- (b) a heavy chain variable region (VHOX40) comprising an amino acid sequence of SEQ ID NO:42 and a light chain variable region (VLOX40) comprising an amino acid sequence of SEQ

- ID NO:43, or
- (c) a heavy chain variable region (VHOX40) comprising an amino acid sequence of SEQ ID NO:44 and a light chain variable region (VLOX40) comprising an amino acid sequence of SEQ ID NO:45, or
- 5 (d) a heavy chain variable region (VHOX40) comprising an amino acid sequence of SEQ ID NO:46 and a light chain variable region (VLOX40) comprising an amino acid sequence of SEQ ID NO:47, or
- (a) a heavy chain variable region (VHOX40) comprising an amino acid sequence of SEQ ID NO:48 and a light chain variable region (VLOX40) comprising an amino acid sequence of SEQ ID NO:49, or
- 10 (a) a heavy chain variable region (VHOX40) comprising an amino acid sequence of SEQ ID NO:50 and a light chain variable region (VLOX40) comprising an amino acid sequence of SEQ ID NO:51, or
- (a) a heavy chain variable region (VHOX40) comprising an amino acid sequence of SEQ ID NO:52 and a light chain variable region (VLOX40) comprising an amino acid sequence of SEQ ID NO:53.

- In one particular aspect, the antigen binding domain capable of specific binding to OX40 comprises (a) a heavy chain variable region (VHOX40) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of
- 20 SEQ ID NO:40, and a light chain variable region (VLOX40) 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:41.

- Particularly, the present invention provides a bispecific antibody, wherein the bispecific antibody comprises
- 25 (a) a fusion polypeptide 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:54, and a fusion polypeptide 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:55,
- (b) a fusion polypeptide comprising an amino acid sequence that is at least about 95%,
- 30 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:56, and a fusion polypeptide 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:57,
- (c) a fusion polypeptide comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:58, and a
- 35 fusion polypeptide comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:59,

- (d) a fusion polypeptide 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:60, and a fusion polypeptide 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:61,
- 5 (e) a fusion polypeptide 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:62, and a fusion polypeptide 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:63,
- (f) a fusion polypeptide 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:64, and a fusion polypeptide 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:65, or
- 10 (g) a fusion polypeptide 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:66, and a fusion polypeptide 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:67.
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Furthermore, the present invention provides a bispecific antibody, wherein the bispecific antibody comprises

- (a) a fusion polypeptide 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:116, and a fusion polypeptide 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:117,
- 20 (b) a fusion polypeptide 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:118, and a fusion polypeptide 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:119,
- 25 (c) a fusion polypeptide 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:120, and a fusion polypeptide 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:121,
- 30 (d) a fusion polypeptide 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:122, and a fusion polypeptide 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:123,
- 35 (e) a fusion polypeptide 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:124, and a

fusion polypeptide 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:125,

(f) a fusion polypeptide 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:126, a fusion
5 polypeptide 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:127, and a light chain that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:128,

(g) a fusion polypeptide comprising an amino acid sequence that is at least about 95%,
10 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:129, and a fusion polypeptide 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:130,

(h) a fusion polypeptide 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:131, and a
15 fusion polypeptide 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:132, or

(i) a fusion polypeptide 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:133, and a fusion
20 polypeptide 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:134.

In some aspects, provided is a bispecific antibody wherein the costimulatory TNF receptor is 4-1BB. In one aspect, provided is a bispecific antibody, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to 4-1BB. Particularly, the bispecific antibody of the invention comprises two antigen
25 binding domains capable of specific binding to 4-1BB.

In some aspects, the antigen binding domain capable of specific binding to 4-1BB a heavy chain variable region (VH4-1BB) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:137, and a light chain variable
30 region (VL4-1BB) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:140. In one aspect, the antigen binding domain capable of specific binding to 4-1BB comprises a heavy chain variable region (VH4-1BB) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or
35 100% identical to the amino acid sequence of SEQ ID NO:141, and a light chain variable region (VL4-1BB) 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:142.

Particularly, the present invention provides a bispecific antibody, wherein the bispecific antibody comprises

- (a) a fusion polypeptide 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:143, and a
5 fusion polypeptide 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:144, or
- (b) a fusion polypeptide 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:145, and a
10 fusion polypeptide 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:146.

In some aspects, provided is a bispecific antibody wherein the costimulatory TNF receptor is CD40. In one aspect, provided is a bispecific antibody, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to CD40. Particularly, the bispecific antibody of the invention comprises two antigen
15 binding domains capable of specific binding to CD40.

In some aspects, the antigen binding domain capable of specific binding to CD40 comprises a heavy chain variable region (VHCD40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:147, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:148, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:149, and
20 a light chain variable region (VLCD40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:150, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:151, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:152. In one aspect, the antigen binding domain capable of specific binding to CD40 comprises a heavy chain variable region (VHCD40) comprising an amino acid sequence that is at least about 95%, 96%,
25 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:153, and a light chain variable region (VLCD40) 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:154.

In another aspect, the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to CD40 comprises
30 (i) a heavy chain variable region (VHCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169 and SEQ ID NO:170, and a light chain variable region (VLCD40) comprising the 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, or
35 (ii) a heavy chain variable region (VHCD40) 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:179 and SEQ ID NO:180, and a light chain variable region (VLCD40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, and SEQ ID NO:184.

Particularly, the present invention provides a bispecific antibody, wherein the bispecific
5 antibody comprises

- (a) a fusion polypeptide 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:155, and a fusion polypeptide 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:156,
- 10 (b) a fusion polypeptide 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:157, and a fusion polypeptide 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:158,
- (c) a fusion polypeptide comprising an amino acid sequence that is at least about 95%,
15 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:159, and a fusion polypeptide 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:160,
- (d) a fusion polypeptide 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:161, and a
20 fusion polypeptide 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:162,
- (e) a fusion polypeptide 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:163, and a fusion polypeptide comprising an amino acid sequence that is at least about 95%, 96%, 97%,
25 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:164, or
- (f) a fusion polypeptide 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:165, and a fusion polypeptide 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:166.

30 The present invention also provides isolated nucleic acid encoding the bispecific antibody of the present invention. Provided is also an expression vector comprising the nucleic acid of the present invention and and furthermore a host cell comprising the isolated nucleic acid or the expression vector of the present invention is provided. Also included is a method of producing a bispecific antibody, comprising culturing the host cell of the present invention under conditions
35 suitable for the expression of the bispecific antibody. The method may also include the step of isolating the bispecific antibody.

The present invention also provides a pharmaceutical composition comprising the bispecific antibody of the present invention and at least one pharmaceutically acceptable excipient.

5 The present invention also provides the bispecific antibody of the present invention, or the pharmaceutical composition of the present invention, for use as a medicament. More particularly, provided is also the bispecific antibody of the invention for use in treating cancer or infectious diseases. In particular, the bispecific antibody of the invention for use in treating cancer is provided.

10 In a further aspect, provided is the use of bispecific antibody of the present invention, or the pharmaceutical composition of the present invention, in the manufacture of a medicament for use

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

The present invention also provides a method of treating an individual having cancer or infectious diseases comprising administering to the individual an effective amount of the
20 bispecific antibody of the present invention, or the pharmaceutical composition of the present invention.

The present invention also provides the use of the bispecific antibody of the present invention, or the pharmaceutical composition of the present invention, in the manufacture of a medicament for up-regulating or prolonging cytotoxic T cell activity. Also provided is a method
25 of up-regulating or prolonging cytotoxic T cell activity in an individual having cancer, comprising administering to the individual an effective amount of the bispecific antibody of the present invention, or the pharmaceutical composition of the present invention. In some embodiments in accordance with various aspects of the present invention the individual is a mammal, particularly a human.

30

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B, 1C and 1D show examples, how the contorsbodies of the invention can be assembled. In **Figure 1A**, the contorsbody consists of a first fusion polypeptide comprising the first half of a fab capable of specific binding to the first target (heavy chain fab) linked via a peptide linker (black line) to a spacer domain linked via peptide linker (black line) to the second

half of the fab capable of specific binding to the first target (light chain fab) which is further linked to a first half of a cross fab capable of specific binding to the second target (VH-Ckappa) (from N to C) and of a second fusion polypeptide comprising the first half of a fab capable of specific binding to the first target (heavy chain fab) linked via a peptide linker (black line) to a spacer domain linked via peptide linker (black line) to the second half of the fab capable of specific binding to the first target (light chain fab) which is further linked to second half of a cross fab capable of specific binding to the second target (VL-CH1) (from N to C). The two spacer domains are different from each other and comprise modifications promoting the association of the first and second fusion polypeptide. An example for this type of contorsbody is CD134-0093 (see Example 2.1). In **Figure 1B**, the contorsbody consists of a first fusion polypeptide comprising the first half of a cross fab capable of specific binding to the first target (VH-Ckappa) linked via a peptide linker (black line) to a spacer domain linked via peptide linker (black line) to the second half of the cross fab capable of specific binding to the first target (VL-CH1) which is further linked to a first half of a fab capable of specific binding to the second target (light chain fab) (from N to C) and of a second fusion polypeptide comprising the first half of a cross fab capable of specific binding to the first target (VH-Ckappa) linked via a peptide linker (black line) to a spacer domain linked via peptide linker (black line) to the second half of the cross fab capable of specific binding to the first target (VL-CH1) which is further linked to second half of the fab capable of specific binding to the second target (heavy chain fab) (from N to C). The two spacer domains are different from each other and comprise modifications promoting the association of the first and second fusion polypeptide. An example for this type of contorsbody is CD134-0094 (see Example 2.2). In **Figure 1C**, the contorsbody consists of a first fusion polypeptide comprising the first half of a fab capable of specific binding to the first target (VL-Ckappa) linked via a peptide linker (black line) to a spacer domain linked via peptide linker (black line) to the second half of the fab capable of specific binding to the first target (VH-CH1) which is further linked to a first half of a cross fab capable of specific binding to the second target (VH-Ckappa) (from N to C) and of a second fusion polypeptide comprising the first half of a fab capable of specific binding to the first target (VL-Ckappa) linked via a peptide linker (black line) to a spacer domain linked via peptide linker (black line) to the second half of the fab capable of specific binding to the first target (VH-CH1) which is further linked to second half of the cross fab capable of specific binding to the second target (VL-CH1) (from N to C). The two spacer domains are different from each other and comprise modifications promoting the association of the first and second fusion polypeptide. An example for this type of contorsbody is P1AE0821 (see Example 2.7). In **Figure 1D**, the contorsbody consists of a first fusion polypeptide comprising the first half of a cross fab capable of specific binding to the second target (VH-Ckappa) linked via a peptide linker (black line) to a first half of a cross fab capable of specific binding to the first target (VH-Ckappa) which is further linked via a peptide linker (black line) to a spacer domain linked via peptide linker (black line) to the second half of the

cross fab capable of specific binding to the first target (VL-CH1) (from N to C) and of a second fusion polypeptide comprising the first half of a cross fab capable of specific binding to the second target (VL-CH1) linked via a peptide linker (black line) to a first half of a cross fab capable of specific binding to the first target (VH-Ckappa) which is further linked via a peptide linker (black line) to a spacer domain linked via peptide linker (black line) to the second half of the cross fab capable of specific binding to the first target (VH-CH1) (from N to C). The two spacer domains are different from each other and comprise modifications promoting the association of the first and second fusion polypeptide. An example for this type of contorsbody is P1AE2735 (see Example 2.14).

10 **Figure 1E** is a schematic drawing of the assembled structure of Contorsbody CD134-0093 (Example 2.1).

 In **Figure 1F** a schematic drawing of the assembled structure of Contorsbody CD134-0094 (Example 2.2) is shown.

Figure 1G is a schematic drawing of the assembled structure of Contorsbodies P1AE0085 and P1AE0086 (Examples 2.3 and 2.4). The antigen binding domain capable of specific binding to the second target (cross fab) is connected via a longer peptide linker that changes the geometry of the molecule.

Figure 1H is a schematic drawing of the assembled structure of Contorsbody P1AE0087 (Example 2.5) and Contorsbody P1AE0839 (Example 2.6). In this case the antigen binding domain capable of specific binding to the second target is a fab and both antigen binding domains capable of specific binding to the first target are cross fabs.

 In **Figure 1I** a schematic drawing of the assembled structure of Contorsbody P1AE0821 (Contorsbody 11, Example 2.7) is shown.

Figure 1J is a schematic drawing of the assembled structure of Contorsbody P1AE1122 (Contorsbody 1, Example 2.8).

 In **Figure 1K** a schematic drawing of the assembled structure of Contorsbody P1AE1887 (Contorsbody 3, Example 2.10) is shown.

 In **Figure 1L** a schematic drawing of the assembled structure of Contorsbody P1AE2254 (Contorsbody 5, Example 2.12) is shown. In the CH and Ckappa fused to the VL and VH of OX40, respectively, amino acid mutations (so-called charged residues) were introduced to prevent the generation of Bence Jones proteins and to further facilitate the correct pairing.

Figure 1M is a schematic drawing of the assembled structure of Contorsbody P1AE2340 (Contorsbody 6, Example 2.13). In this case the molecule is composed of two fusion proteins and a light chain.

35 **Figure 1N** is a schematic drawing of the assembled structure of Contorsbody P1AE2735 (Contorsbody 8, Example 2.14).

Figure 1O is a schematic drawing of the 2+1 OX40 x FAP bispecific antibody with bivalent binding for OX40 and monovalent binding for FAP (positive control molecule). A negative control molecule with the same structure was made wherein the FAP binding domain was replaced by DP47 germline (2+1 OX40 x DP47 antibody).

- 5 **Figure 1P** is a schematic drawing of the 4+1 OX40 x FAP bispecific antibody with tetravalent binding for OX40 and monovalent binding for FAP. These control molecules are described in more detail in Example 2.18.

In **Figures 2A, 2B, 2C and 2D** is shown the binding of OX40 x FAP bispecific antibodies to activated CD4⁺ and CD8⁺ T-cells (**Figures 2A** and **2C**, respectively) and resting CD4⁺ and CD8⁺ T-cells (**Figures 2B** and **2D**, respectively). The tetravalent 4+1 OX40 x FAP (4B9) bispecific antibody showed the strongest binding to CD4⁺ and CD8⁺ T-cells. The 2+1 formats 2+1 OX40 (49B4) x FAP (28H1) and 2+1 OX40 (49B4) x DP47 showed intermediate binding. The Contorsbody CD134-0093 indicated stronger binding than the 2+1 formats, whereas the Contorsbody CD134-0094 bound less strong. Binding to CD4⁺ T-cells was much stronger than that to CD8⁺ T cells. The negative control DP47 hu IgG1 P329G LALA did not bind to CD4⁺ or CD8⁺ T-cells. None of the molecules bound to resting CD4⁺ or CD8⁺ T-cells (**Figures 2B** and **2D**).

Figures 2E, 2F, 2G and 2H show the results as obtained with Contorsbodies P1AE0085, P1AE0086 and P1AE0087. All 2+1 formats OX40 (49B4) x FAP (28H1), OX40 (49B4) x FAP (4B9) and OX40 (49B4) x DP47 showed a similarly good binding to activated CD4⁺ and CD8⁺ T-cells (**Figure 2E** and **2G**, respectively) whereas the binding to OX40 was partially impaired for the Contorsbody molecules, especially for the Contorsbody P1AE0087. DP47 hu IgG1 P329G LALA did not bind to CD4⁺ or CD8⁺ T-cells as expected. No binding was observed to resting CD4⁺ or CD8⁺ T-cells (**Figure 2F** and **2H**, respectively) for any of the tested molecules.

25 The binding to human FAP-expressing tumor cells is shown in **Figures 3A, 3B, 3C and 3D**. In a first experiment, Contorsbodies CD134-0093 and CD134-0094 were compared with the control molecules. Binding to WM266-4 (FAP⁺ positive) and A549NLR (FAP negative) tumor cells is shown in **Figures 3A** and **3B**, respectively. All OX40 x FAP bispecific antibodies bound efficiently to human FAP expressing target cells. The tetravalent 4+1 OX40 x FAP (4B9, high affinity to FAP) bispecific antibody bound strongest to FAP⁺ cells, followed by the Contorsbody CD134-0093, Contorsbody CD1334-0094 and the 2+1 OX40 (49B4) x FAP (28H1) bispecific antibody. The non-targeted 2+1 OX40 (49B4) x DP47 and the negative control (DP47 hu IgG1 P329G LALA) did not bind to any FAP⁺ cells. **Figures 3C** and **3D** show the binding of Contorsbodies P1AE0085, P1AE0086 and P1AE0087 to NIH/3T3huFAP clone 19 (FAP⁺) (Figure 3C) and A549NLR (FAP⁻) tumor cells (Figure 3D). All FAP-targeted anti OX40 antibodies bound efficiently to human FAP expressing target cells. The binding of the

Contorsbody molecules was slightly impaired as compared to their respective controls. Again, the non-targeted 2+1 OX40 (49B4) x DP47 and the negative control (DP47 hu IgG1 P329G LALA) showed no binding to FAP⁺ cells.

In **Figures 4A, 4B, 4C, 4D, 4E and 4F** the NFκB activation with different types of cross-linking is shown. Using FAP expressing cells (NIH/3T3 huFAP clone 19) as crosslinkers, the FAP targeted 2+1 OX40 (49B4) x FAP (28H1) construct induced the strongest NFκB activation, followed by Contorsbodies CD134-0093 and CD134-0094, whereas CD134-0094 showed a slightly stronger activation than CD134-0093. The non-targeted and therefore not crosslinked 2 non-targeted 2+1 OX40 (49B4) x DP47 induced weak NFκB activation (**Figure 4A**). When using a secondary, anti hu IgG1 Fcγ-specific antibody as crosslinker, the two 2+1 constructs OX40 (49B4) x FAP (28H1) and OX40 (49B4) x DP47 behaved similar. The Contorsbodies CD134-0093 and CD134-0094 ran similar as well, but lower than the 2+1 constructs (**Figure 4B**). The least NFκB activation was obtained by not using crosslinkers. The 2+1 constructs OX40 (49B4) x FAP (28H1) and OX40 (49B4) x DP47 showed a moderate NFκB activation, followed by the even less potent Contorsbodies CD134-0093 and CD134-0094. DP47 hu IgG1 P329G LALA did not induce any NFκB activation (**Figure 4C**). The ability of Contorsbodies P1AE0085, P1AE0086 and P1AE0087 to induce NFκB activation with different types of cross-linking is shown in **Figures 4D to 4F**. In the absence of cross-linking, only a weak signal could be detected at the highest antibody concentration (**Figure 4D**). The three Contorsbody molecules induced a very similar NFκB activation to that of FAP-targeted 2+1 formats OX40 (49B4) x FAP (28H1) and OX40 (49B4) x FAP (4B9) when cross-linked by human FAP-expressing cells (**Figure 4E**). In the presence of secondary antibody cross-linking, all three Contorsbody molecules displayed slightly lower NFκB activation than the 2+1 control molecules (**Figure 4F**). DP47 hu IgG1 P329G LALA did not induce any NFκB activation.

Ox40 mediated co-stimulation of sub-optimally TCR triggered resting human PBMCs and hyper-crosslinking by cell surface FAP is shown in **Figures 5A, 5B, 5C, 5D, 5E and 5F**. **Figures 5A and 5B** show the FSC-A ("area" of a Forward Side Scatter (FSC) pulse), respectively the size of CD4 and CD8 T-cells, respectively, after suboptimal CD3 stimulation as measured as intensity of light scattered at small angles by FACS analysis. The FAP targeted 2+1 construct OX40 (49B4) x FAP (28H1) showed an intermediate increase in size, whereas the Contorsbody CD134-0093 indicated a stronger increase. The untargeted 2+1 OX40 (49B4) x DP47, the Contorsbody CD134-0094 and the negative control (DP47 hu IgG1 P329G LALA) did not change the size of neither CD4 nor CD8 T-cells. **Figures 5C and 5D** show the activation of CD4 and CD8 T-cells using the surface marker CD25. For the CD4 T-cells (**Figure 5C**) contorsbodies CD134-0093 and CD134-0094 demonstrated the highest activation, followed by the somehow less potent targeted 2+1 OX40 (49B4) x FAP (28H1). The untargeted OX40 (49B4) x DP47 and the negative control did not show any activation after baseline-correction. For the

CD8 T-cells (**Figure 5D**), the Contorsbody CD134-0094 demonstrated a stronger activation compared to the 2+1 OX40 (49B4) x FAP (28H1) and CD134-0093. **Figures 5E and 5F** show the upon activation downregulated IL-7R α (CD127). The Contorsbody CD134-0093 showed the strongest CD127 downregulation for CD4 and CD8 T-cells, followed by the targeted 2+1 OX40 (49B4) x FAP (28H1) and the Contorsbody CD134-0094.

Figure 6 illustrates the normalized area under the curve (AUC) values for FSC-A and CD25 on CD4 and CD8 T-cells. The filled symbols represent CD4 T-cells, the empty one CD8 T cells. All values were normalized to the top AUC values of the Contorsbody CD134-0093 (= 100%). The untargeted 2+1 construct OX40 (49B4) x DP47 showed only minimal activation on CD4 and CD8 T cells, whereas the FAP targeted 2+1 construct OX40 (49B4) x FAP (28H1) demonstrated a higher potency (normalized values between 60 and 100) regarding FSC-A and CD25 on CD4 and CD8 T cells, but less potency compared to CD134-0093. For the Contorsbody CD134-0094, only the CD25 MFI AUC values of CD4 and CD8 T-cells showed similar activation compared to CD134-0093.

The effects of Contorsbodies P1AE0085, P1AE0086 and P1AE0087 on OX40 mediated co-stimulation of sub-optimally TCR triggered PBMCs and hyper-crosslinking by cell surface FAP are shown in **Figures 7A, 7B, 7C and 7D**. **Figures 7A and 7B** show the FSC-A, respectively the size of CD4 and CD8 T cells, respectively, after suboptimal CD3 stimulation. **Figures 7C and 7D** show the activation of CD4 and CD8 T-cells, respectively, using the expression of the surface marker CD25. All FAP-targeted molecules (controls and all three contorsbody molecules) induced a dose-dependent increase in the Forward Side Scatter Area and CD25 expression on both CD4 and CD8 T cells. The untargeted 2+1 construct OX40 (49B4) x DP47 and the negative control (DP47 hu IgG1 P329G LALA) did not show any activation after baseline-correction.

In **Figures 8A, 8B, 8C and 8D** is shown the binding of different OX40 x FAP contorsbodies to activated CD4⁺ T-cells. The untargeted 2+1 construct OX40 (49B4) x DP47 was used as positive control and DP47 hu IgG1 P329G LALA as negative control. **Fig. 8A** shows the binding of Contorsbody 1 (P1AE1122), Contorsbody 2 (P1AE1942) and Contorsbody 3 (P1AE1887) and **Fig. 8B** shows the binding of Contorsbody 4 (P1AE1888), Contorsbody 5 (P1AE2254) and Contorsbody 6 (P1AE2340). The binding of Contorsbody 7 (P1AE0086) and Contorsbody 8 (P1AE2735) is shown in **Fig. 8C** and the binding of Contorsbody 9 (P1AE2743) and Contorsbody 10 (P1AE2762) is shown in **Fig. 8D**. In **Figures 9A, 9B, 9C and 9D**, respectively, it is shown that none of the tested Contorsbodies bound to resting CD4 T cells. In **Figures 10A, 10B, 10C and 10D** is shown the binding of the same OX40 x FAP contorsbodies to activated CD8⁺ T-cells. The untargeted 2+1 construct OX40 (49B4) x DP47 was used as positive control and DP47 hu IgG1 P329G LALA as negative control. In **Figures 11A, 11B, 11C**

and 11D, respectively, it is shown that none of the tested Contorsbodies bound to resting CD8 T cells. A summary of the area under the curve values for the binding to activated or resting CD4⁺ T-cells is shown in **Figure 12A** and for the binding to activated or resting CD8⁺ T cell in **Figure 12B**.

5 The binding to human FAP-expressing tumor cells of Contorsbodies 1 to 10 is shown in **Figures 13A, 13B, 13C and 13D** and **Figures 14A, 14B, 14C and 14D**, respectively. Binding to NIH/3T3-huFAP clone 19 (FAP⁺) tumor cells (FAP⁺ positive) is shown in **Figures 12A to 12D**, respectively. **Fig. 13A** shows the binding of Contorsbody 1 (P1AE1122), Contorsbody 2 (P1AE1942) and Contorsbody 3 (P1AE1887) and **Fig. 13B** shows the binding of Contorsbody 4 (P1AE1888), Contorsbody 5 (P1AE2254) and Contorsbody 6 (P1AE2340). The binding of Contorsbody 7 (P1AE0086) and Contorsbody 8 (P1AE2735) is shown in **Fig. 13C** and the binding of Contorsbody 9 (P1AE2743) and Contorsbody 10 (P1AE2762) is shown in **Fig. 13D**. All OX40 x FAP bispecific contorsbodies bound efficiently to human FAP expressing target cells. Contorsbody 8 (Fig. 12C) bound strongest to FAP⁺ cells, followed by Contorsbody 10 (Fig. 12D) and Contorsbody 6 (Fig. 12B), much stronger than the 2+1 bispecific antibody OX40 (49B4) x FAP (4B9). The untargeted 2+1 construct OX40 (49B4) x DP47 and the negative control (DP47 hu IgG1 P329G LALA) did not bind to any FAP⁺ cells. The corresponding binding of Contorsbodies 1 to 10 to A549NLR (FAP⁻ negative) tumor cells is shown in **Figures 14A to 14D**. None of the FAP-targeted molecules (Contorsbodies 1 to 10 or 2+1 bispecific antibody OX40 (49B4) x FAP (4B9)) were able to bind to FAP⁻ target cells.

In **Figures 15A, 15B, 15C and 15D** the NFκB activation of Contorsbodies 1 to 11 with cross-linking is shown. Using FAP expressing cells (NIH/3T3 huFAP clone 19) as crosslinkers, the NFκB activation was comparable for all of the contorsbodies, with Contorsbody 11 (P1AE0821) being the least potent. The non-targeted and therefore not crosslinked 2 non-targeted 2+1 OX40 (49B4) x DP47 antibody (7718) induced weak NFκB activation, whereas bispecific OX40 (49B4) x FAP (4B9) antibody (7719) caused the highest NFκB activation. DP47 hu IgG1 P329G LALA (8105) did not induce any NFκB activation. NFκB activation without cross-linking by FAP is shown in **Figures 16A, 16B, 16C and 16D**. In the absence of cross-linking, only a weak signal could be detected at the higher antibody concentrations. The non-targeted 2+1 OX40 (49B4) x DP47 antibody (7718) showed a moderate NFκB activation, followed by the Contorsbody 7 (Fig 16C). The other contorsbodies induced minor or zero levels. The negative control (8105) did not induce any NFκB activation. Normalized Area under the curve values for NFκB activation in HeLa cells with and without crosslinking with FAP⁺ cells are summarized in **Figure 17**. The negative control (8105) did not induce any NFκB activation in both cases, while non-targeted 2+1 OX40 (49B4) x DP47 antibody (7718) showed only minimal activation in both cases. Bispecific OX40 (49B4) x FAP (4B9) antibody (7719)

demonstrated the higher levels of activation, followed by Contorbodies 8, 9 and 10. Lower levels were observed for Contorboby 11 (P1AE0821).

OX40 mediated co-stimulation of sub-optimally TCR triggered resting human PBMCs and hyper-crosslinking by cell surface FAP of Contorsbodies 7, 8, 9 and 10 is shown in **Figures 18A, 18B, 18C and 18D**. Figures 18A and 18B show the activation of CD4 T cells and Figures 18C and 18D show the activation of CD8 T cells using the surface marker CD25 after suboptimal CD3 stimulation. For the CD4 T-cells (Figures 18A nad 18B), the bispecific OX40 (49B4) x FAP (4B9) antibody (7719) demonstrated the highest activation, followed by the minimally less potent Contorboby 8. The non-targeted 2+1 OX40 (49B4) x DP47 antibody (7718) and the negative control (8105) did not show any activation after baseline-correction. For the CD8 T-cells (Figures 18C and 18D), the Contorbodies 7, 8 and 9 as well as the bispecific OX40 (49B4) x FAP (4B9) antibody (7719) demonstrated a stronger activation compared to Contorboby 10. **Figures 19A, 19B, 19C and 19D** show the FSC-A, respectively the size of CD4 (Figures 19A and 19B) and CD8 T-cells (Figures 19C and 19D) after suboptimal CD3 stimulation. All contorbodies indicated a comparable intermediate increase. Addition of the untargeted 2+1 anti OX40 molecule (7718) and the negative control (8105) did not change the size of neither CD4 nor CD8 T-cells. **Figures 20A, 20B, 20C and 20D** show the eFluor 670 levels, respectively the proliferation of CD4 (Figures 20A and 20B) and CD8 T-cells (Figures 20C and 20D) after suboptimal CD3 stimulation. In CD4 subpopulations, Contorbodies 8 and 10 showed a considerable decrease in eFluor 670 levels, indicating stronger proliferation. In CD8 subpopulations Contorbodies 7 and 9 showed bigger decrease in eFluor 670 levels. The untargeted 2+1 anti OX40 molecule (7718) and the negative control (8105) showed only a minor decrease in all subpopulations. **Figures 21A, 21B, 21C and 21D** show the upon activation downregulated IL-7R α (CD127). The the bispecific OX40 (49B4) x FAP (4B9) antibody (7719) showed the strongest downregulation for CD4 and CD8 T-cells, followed by Contorboby 10. Greater downregulation of CD127 was observed in CD4 cells than CD8 subpopulations. Normalized Area under the curve values for CD25 (Fig. 22A), FSC-A (Fig. 22B), eFluor 670 (Fig. 22C) and CD127 (FIG. 22D) on CD4 and CD8 T-cells are summarized in **Figures 22A, 22B, 22C and 22D**. The untargeted 2+1 anti OX40 molecule (7718) showed only minimal activation on CD4 and CD8 T cells, whereas the bispecific OX40 (49B4) x FAP (4B9) antibody (7719) demonstrated a higher potency regarding eFluor 679 and CD127 on CD4 and CD8 T-cells compared to the contorbodies. CD25 and FSC-A indicated similar activation levels in both the 7719 molecule and contorbodies in both CD4 and CD8 subpopulations.

Figures 23A and 23B show the NF κ B-mediated luciferase expression activity in 4-1BB expressing reporter cell line Jurkat-hu4-1BB-NF κ B-luc2. To test the functionality of 2+1 anti-4-1BB (20H4.9) x anti-FAP(4B9) contorsbodies versus 2+1 anti-4-1BB (20H4.9) x anti-FAP (4B9) antigen binding molecules versus controls, molecules were incubated. The concentration of

antigen binding molecules or its controls are blotted against the units of released light (RLU) measured after 6 h of incubation. All values are baseline corrected by subtracting the baseline values of the blank control (e.g. no antibodies added). In **Figure 23A** FAP-target-independent 4-1BB activation is shown, whereby 4-1BB-binding induces NFκB-controlled luciferase expression in the reporter cell line without any FAP-mediated crosslinking. In **Figure 23B** high FAP-expressing cell line NIH/3T3-huFAP clone 19 (human-FAP-transgenic mouse fibroblast cell line) was added. The FAP-expressing tumor cells lead to crosslinking of the bispecific 4-1BB (20H4.9) x FAP (4B9) antigen binding molecules and a strong increase of its potential to induce NFκB-induced /luciferase activation in the 4-1BB-expressing reporter cell line. The bispecific 2+1 anti-4-1BB (20H4.9) x anti-FAP(4B9) antigen binding molecules (black filled star and line) showed a slightly better activation (lower EC50 values) than the contorsbodies. However, the activation caused by the contorsbodies was much higher the activation shown by the untargeted 4-1BB antibodies.

Figures 24A, 24B, 24C, 24D, 24E, 24F, 24G and 24H show the *in vitro* activation of human B cells by bivalent human anti-CD40 x FAP contorsbodies in the presence of FAP-coated (Fig. 24A, 24C, 24E and 24G) or uncoated Dynabeads® (Fig. 24B, 24D, 24F and 24H) after 2 days incubation. Compared to the FAP-independent upregulation of CD69, CD80, CD86 and HLA-DR induced by cross-linked SGN40, upregulation of these activation markers induced by FAP-dependent bispecific antigen binding molecules in the presence of FAP-coated beads was slightly lower. In the absence of FAP (uncoated beads) no increase of CD69, CD80, CD86 or HLA-DR expression could be observed with the FAP-targeted anti-CD40 contorsbodies, while the cross-linked positive control antibody SGN40 induced an upregulation of these activation markers. Shown is the percentage of CD69, CD80, CD86 or HLA-DR positive vital B cells after 2 days of incubation with the indicated titrated contorsbodies or control antibody. XL stands for cross-linking with F(ab')₂ Fragment Goat anti-human IgG Fcγ fragment specific. The x-axis shows the concentration of contorsbody constructs or the control antibody.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as generally used in the art to which this invention belongs. For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa.

As used herein, the term "**antigen binding molecule**" refers in its broadest sense to a molecule that specifically binds an antigenic determinant. Examples of antigen binding molecules are antibodies, antibody fragments and scaffold antigen binding proteins.

The term "**antigen binding domain**" refers to the part of an antigen binding molecule that specifically binds to an antigenic determinant. In one aspect, the antigen binding domain is able to activate signaling through its target cell antigen. Antigen binding domains include the area or fragment of an antibody which specifically binds to and is complementary to part or all of an antigen. In addition, antigen binding domains include scaffold antigen binding proteins as further defined herein, e.g. binding domains which are based on designed repeat proteins or designed repeat domains (see e.g. WO 2002/020565). In particular, an antigen binding domain is comprised of a first part and a second part, wherein the first part comprises an antibody light chain variable region (VL) and the second part comprises an antibody heavy chain variable region (VH) or vice versa.

The term "**antibody**" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, monospecific and multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

The term "**monoclonal antibody**" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g. containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen.

The term "**monospecific**" antibody as used herein denotes an antibody that has one or more binding sites each of which bind to the same epitope of the same antigen. The term "**bispecific**" means that the antigen binding molecule is able to specifically bind to at least two distinct antigenic determinants. A bispecific antigen binding molecule comprises at least two antigen binding sites, each of which is specific for a different antigenic determinant. In certain embodiments the bispecific antigen binding molecule is capable of simultaneously binding two antigenic determinants, particularly two antigenic determinants expressed on two distinct cells. For example, the antigen binding molecules of the present invention are bispecific, comprising an antigen binding domain capable of specific binding to a first target, and an antigen binding domain capable of specific binding to a second target. In one particular aspect, the antibody of

the present invention comprises an antigen binding domain capable of specific binding to OX40 and an antigen binding domain capable of specific binding to FAP. In another particular aspect, the antibody of the present invention comprises an antigen binding domain capable of specific binding to 4-1BB and an antigen binding domain capable of specific binding to FAP. In one
5 further aspect, the antibody of the present invention comprises an antigen binding domain capable of specific binding to CD40 and an antigen binding domain capable of specific binding to FAP.

The term “**valent**” as used within the current application denotes the presence of a specified number of binding sites in an antigen binding molecule. As such, the terms “bivalent”,
10 “tetravalent”, and “hexavalent” denote the presence of two binding sites, four binding sites, and six binding sites, respectively, in an antigen binding molecule. Valency of an antigen binding molecule may also be expressed in relation to the number of binding sites for a given antigenic determinant. For example, the bispecific antibodies of the present invention are bivalent with respect to a first target, and monovalent with respect to a second target.

15 The terms “full length antibody”, “intact antibody”, and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure. “**Native antibodies**” refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG-class antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that
20 are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a light chain constant domain (CL), also called a light
25 chain constant region. The heavy chain of an antibody may be assigned to one of five types, called α (IgA), δ (IgD), ϵ (IgE), γ (IgG), or μ (IgM), some of which may be further divided into subtypes, e.g. $\gamma 1$ (IgG1), $\gamma 2$ (IgG2), $\gamma 3$ (IgG3), $\gamma 4$ (IgG4), $\alpha 1$ (IgA1) and $\alpha 2$ (IgA2). The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

30 An “**antibody fragment**” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies, triabodies, tetrabodies, cross-Fab fragments; linear antibodies; single-chain antibody molecules (e.g. scFv); and single domain antibodies. For a review of certain antibody fragments,
35 see Hudson et al., Nat Med 9, 129-134 (2003). For a review of scFv fragments, see e.g. Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore

eds., Springer-Verlag, New York, pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific, see, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

Papain digestion of intact antibodies produces two identical antigen-binding fragments, called "Fab" fragments containing each the heavy- and light-chain variable domains and also the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. As used herein, Thus, the term "**Fab fragment**" refers to an antibody fragment comprising a light chain fragment comprising a VL and a constant domain of a light chain (CL), and a VH and a first constant domain (CH1) of a heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteins from the antibody hinge region. Fab'-SH are Fab' fragments wherein the cysteine residue(s) of the constant domains bear a free thiol group. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites (two Fab fragments) and a part of the Fc region. According to the present invention, the term "Fab fragment" also includes "cross-Fab fragments" or "crossover Fab fragments" as defined below.

The term "**cross-Fab fragment**" or "xFab fragment" or "crossover Fab fragment" refers to a Fab fragment, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged. Two different chain compositions of a cross-Fab molecule are possible and comprised in the bispecific antibodies of the invention: On the one hand, the variable regions of the Fab heavy and light chain are exchanged, i.e. the crossover Fab molecule comprises a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1), and a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL). This crossover Fab molecule is also referred to as CrossFab_(VLVH). On the other hand, when the constant regions of the Fab heavy and light chain are exchanged, the crossover Fab molecule comprises a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL), and a peptide chain composed of the light chain

variable region (VL) and the heavy chain constant region (CH1). This crossover Fab molecule is also referred to as CrossFab_(CLCH1).

A “single chain Fab fragment” or “**scFab**” is a polypeptide consisting of an antibody heavy chain variable domain (VH), an antibody constant domain 1 (CH1), an antibody light chain variable domain (VL), an antibody light chain constant domain (CL) and a linker, wherein said antibody domains and said linker have one of the following orders in N-terminal to C-terminal direction: a) VH-CH1-linker-VL-CL, b) VL-CL-linker-VH-CH1, c) VH-CL-linker-VL-CH1 or d) VL-CH1-linker-VH-CL; and wherein said linker is a polypeptide of at least 30 amino acids, preferably between 32 and 50 amino acids. Said single chain Fab fragments are stabilized via the natural disulfide bond between the CL domain and the CH1 domain. In addition, these single chain Fab molecules might be further stabilized by generation of interchain disulfide bonds via insertion of cysteine residues (e.g. position 44 in the variable heavy chain and position 100 in the variable light chain according to Kabat numbering).

A “crossover single chain Fab fragment” or “**x-scFab**” is a polypeptide consisting of an antibody heavy chain variable domain (VH), an antibody constant domain 1 (CH1), an antibody light chain variable domain (VL), an antibody light chain constant domain (CL) and a linker, wherein said antibody domains and said linker have one of the following orders in N-terminal to C-terminal direction: a) VH-CL-linker-VL-CH1 and b) VL-CH1-linker-VH-CL; wherein VH and VL form together an antigen-binding site which binds specifically to an antigen and wherein said linker is a polypeptide of at least 30 amino acids. In addition, these x-scFab molecules might be further stabilized by generation of interchain disulfide bonds via insertion of cysteine residues (e.g. position 44 in the variable heavy chain and position 100 in the variable light chain according to Kabat numbering).

A “**single-chain variable fragment (scFv)**” is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of an antibody, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or *vice versa*. This protein retains the specificity of the original antibody, despite removal of the constant regions and the introduction of the linker. scFv antibodies are, e.g. described in Houston, J.S., Methods in Enzymol. 203 (1991) 46-96). In addition, antibody fragments comprise single chain polypeptides having the characteristics of a VH, namely being able to assemble together with a VL, or of a VL, namely being able to assemble together with a VH to a functional antigen binding site and thereby providing the antigen binding property of full length antibodies.

“**Scaffold antigen binding proteins**” are known in the art, for example, fibronectin and designed ankyrin repeat proteins (DARPs) have been used as alternative scaffolds for antigen-binding domains, see, e.g., Gebauer and Skerra, Engineered protein scaffolds as next-generation antibody therapeutics. *Curr Opin Chem Biol* 13:245-255 (2009) and Stumpp et al., Darpins: A new generation of protein therapeutics. *Drug Discovery Today* 13: 695-701 (2008). In one aspect of the invention, a scaffold antigen binding protein is selected from the group consisting of CTLA-4 (Evibody), Lipocalins (Anticalin), a Protein A-derived molecule such as Z-domain of Protein A (Affibody), an A-domain (Avimer/Maxibody), a serum transferrin (*trans*-body); a designed ankyrin repeat protein (DARPin), a variable domain of antibody light chain or heavy chain (single-domain antibody, sdAb), a variable domain of antibody heavy chain (nanobody, aVH), V_{NAR} fragments, a fibronectin (AdNectin), a C-type lectin domain (Tetranectin); a variable domain of a new antigen receptor beta-lactamase (V_{NAR} fragments), a human gamma-crystallin or ubiquitin (Affilin molecules); a kunitz type domain of human protease inhibitors, microbodies such as the proteins from the knottin family, peptide aptamers and fibronectin (adnectin). CTLA-4 (Cytotoxic T Lymphocyte-associated Antigen 4) is a CD28-family receptor expressed on mainly CD4⁺ T-cells. Its extracellular domain has a variable domain- like Ig fold. Loops corresponding to CDRs of antibodies can be substituted with heterologous sequence to confer different binding properties. CTLA-4 molecules engineered to have different binding specificities are also known as Evibodies (e.g. US7166697B1). Evibodies are around the same size as the isolated variable region of an antibody (e.g. a domain antibody). For further details see *Journal of Immunological Methods* 248 (1-2), 31-45 (2001). Lipocalins are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids. They have a rigid beta-sheet secondary structure with a number of loops at the open end of the conical structure which can be engineered to bind to different target antigens. Anticalins are between 160-180 amino acids in size, and are derived from lipocalins. For further details see *Biochim Biophys Acta* 1482: 337-350 (2000), US7250297B1 and US20070224633. An affibody is a scaffold derived from Protein A of *Staphylococcus aureus* which can be engineered to bind to antigen. The domain consists of a three-helical bundle of approximately 58 amino acids. Libraries have been generated by randomization of surface residues. For further details see *Protein Eng. Des. Sel.* 2004, 17, 455-462 and EP 1641818A1. Avimers are multidomain proteins derived from the A-domain scaffold family. The native domains of approximately 35 amino acids adopt a defined disulfide bonded structure. Diversity is generated by shuffling of the natural variation exhibited by the family of A-domains. For further details see *Nature Biotechnology* 23(12), 1556 - 1561 (2005) and *Expert Opinion on Investigational Drugs* 16(6), 909-917 (June 2007). A transferrin is a monomeric serum transport glycoprotein. Transferrins can be engineered to bind different target antigens by insertion of peptide sequences in a permissive surface loop. Examples of engineered transferrin scaffolds include the *Trans-body*. For further details see *J. Biol. Chem* 274, 24066-24073 (1999). Designed Ankyrin Repeat

Proteins (DARPin) are derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is a 33 residue motif consisting of two alpha-helices and a beta-turn. They can be engineered to bind different target antigens by randomizing residues in the first alpha-helix and a beta-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details see J. Mol. Biol. 332, 489-503 (2003), PNAS 100(4), 1700-1705 (2003) and J. Mol. Biol. 369, 1015-1028 (2007) and US20040132028A1. A single-domain antibody is an antibody fragment consisting of a single monomeric variable antibody domain. The first single domains were derived from the variable domain of the antibody heavy chain from camelids (nanobodies or V_HH fragments). Furthermore, the term single-domain antibody includes an autonomous human heavy chain variable domain (aVH) or V_{NAR} fragments derived from sharks. Fibronectin is a scaffold which can be engineered to bind to antigen. Adnectins consists of a backbone of the natural amino acid sequence of the 10th domain of the 15 repeating units of human fibronectin type III (FN3). Three loops at one end of the .beta.- sandwich can be engineered to enable an Adnectin to specifically recognize a therapeutic target of interest. For further details see Protein Eng. Des. Sel. 18, 435- 444 (2005), US20080139791, WO2005056764 and US6818418B1. Peptide aptamers are combinatorial recognition molecules that consist of a constant scaffold protein, typically thioredoxin (TrxA) which contains a constrained variable peptide loop inserted at the active site. For further details see Expert Opin. Biol. Ther. 5, 783-797 (2005). Microbodies are derived from naturally occurring microproteins of 25-50 amino acids in length which contain 3-4 cysteine bridges - examples of microproteins include KalataBI and conotoxin and knottins. The microproteins have a loop which can be engineered to include upto 25 amino acids without affecting the overall fold of the microprotein. For further details of engineered knottin domains, see WO2008098796.

25 An “**antigen binding molecule that binds to the same epitope**” as a reference molecule refers to an antigen binding molecule that blocks binding of the reference molecule to its antigen in a competition assay by 50% or more, and conversely, the reference molecule blocks binding of the antigen binding molecule to its antigen in a competition assay by 50% or more.

As used herein, the term “**antigenic determinant**” is synonymous with “antigen” and “epitope,” and refers to a site (e.g. a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antigen binding moiety binds, forming an antigen binding moiety-antigen complex. Useful antigenic determinants can be found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, on the surface of immune cells, free in blood serum, and/or in the extracellular matrix (ECM). The proteins useful as antigens herein can be any native form the proteins from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g. mice and rats), unless

otherwise indicated. In a particular embodiment the antigen is a human protein. Where reference is made to a specific protein herein, the term encompasses the “full-length”, unprocessed protein as well as any form of the protein that results from processing in the cell. The term also encompasses naturally occurring variants of the protein, e.g. splice variants or allelic variants.

5 The term "**paratope**" refers to that part of a given antibody molecule that is required for specific binding between a target and a binding site. A paratope may be continuous, i.e. formed by adjacent amino acid residues present in the binding site, or discontinuous, i.e. formed by amino acid residues that are at different positions in the primary sequence of the amino acid residues, such as in the amino acid sequence of the CDRs of the amino acid residues, but in close
10 proximity in the three-dimensional structure, which the binding site adopts.

By "**specific binding**" is meant that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The ability of an antigen binding molecule to bind to a specific antigen can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. Surface
15 Plasmon Resonance (SPR) technique (analyzed on a BIAcore instrument) (Liljeblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)). In one embodiment, the extent of binding of an antigen binding molecule to an unrelated protein is less than about 10% of the binding of the antigen binding molecule to the antigen as measured, e.g. by SPR. In certain embodiments, a molecule that binds to the antigen has a dissociation
20 constant (K_d) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g. from 10^{-9} M to 10^{-13} M).

"**Affinity**" or "binding affinity" refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g. an antibody) and its binding partner (e.g. an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic
25 binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g. antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d), which is the ratio of dissociation and association rate constants (k_{off} and k_{on} , respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by common
30 methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR).

An "**affinity matured**" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for
35 antigen.

A “**tumor associated antigen**” as used herein refers to an antigenic determinant presented on the surface of a target cell, which is a cell in a tumor such as a cancer cell, a cell of the tumor stroma or a B cell. In certain aspects, the tumor associated antigen is Fibroblast Activation Protein (FAP).

5 The term “**capable of specific binding to Fibroblast activation protein (FAP)**” refers to an antigen binding molecule that is capable of binding FAP with sufficient affinity such that the antigen binding molecule is useful as a diagnostic and/or therapeutic agent in targeting FAP. The antigen binding molecule includes but is not limited to, antibodies, Fab molecules, crossover Fab molecules, single chain Fab molecules, Fv molecules, scFv molecules, single domain antibodies,
10 and VH and scaffold antigen binding protein. In one aspect, the extent of binding of an anti-FAP antigen binding molecule to an unrelated, non-FAP protein is less than about 10% of the binding of the antigen binding molecule to FAP as measured, e.g., by Surface Plasmon Resonance (SPR). In particular, an antigen binding molecule that is capable of specific binding to FAP has a dissociation constant (K_d) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or
15 $\leq 0.001 \text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-FAP antigen binding molecule binds to FAP from different species. In particular, the anti-FAP antigen binding molecule binds to human, cynomolgus and mouse FAP.

 The term “**Fibroblast activation protein (FAP)**”, also known as Prolyl endopeptidase
20 FAP or Seprase (EC 3.4.21), refers to any native FAP from any vertebrate source, including mammals such as primates (e.g. humans) non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed FAP as well as any form of FAP which results from processing in the cell. The term also encompasses naturally occurring variants of FAP, e.g., splice variants or allelic variants. In
25 one embodiment, the antigen binding molecule of the invention is capable of specific binding to human, mouse and/or cynomolgus FAP. The amino acid sequence of human FAP is shown in UniProt (www.uniprot.org) accession no. Q12884 (version 149, SEQ ID NO:97), or NCBI (www.ncbi.nlm.nih.gov/) RefSeq NP_004451.2. The extracellular domain (ECD) of human FAP extends from amino acid position 26 to 760. The amino acid sequence of mouse FAP is shown in
30 UniProt accession no. P97321 (version 126, SEQ ID NO:98), or NCBI RefSeq NP_032012.1. The extracellular domain (ECD) of mouse FAP extends from amino acid position 26 to 761. Preferably, an anti-FAP binding molecule of the invention binds to the extracellular domain of FAP. Exemplary anti-FAP binding molecules are described in International Patent Application No. WO 2012/020006 A2.

35 The term “**variable region**” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antigen binding molecule to antigen. The

variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL may be
5 sufficient to confer antigen-binding specificity.

The term “**hypervariable region**” or “HVR” as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence (“complementarity determining regions” or “**CDRs**”) and/or form structurally defined loops (“hypervariable loops”) and/or contain the antigen-contacting residues (“antigen contacts”). Generally, antibodies
10 comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

- (a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));
- 15 (b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));
- (c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996));
20 and
- (d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

Unless otherwise indicated, HVR (e.g. CDR) residues and other residues in the variable
25 domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

Kabat *et al.* also defined a numbering system for variable region sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of “Kabat numbering” to any variable region sequence, without reliance on any experimental data beyond the sequence itself. As used herein, “Kabat numbering” refers to the numbering
30 system set forth by Kabat et al., U.S. Dept. of Health and Human Services, “Sequence of Proteins of Immunological Interest” (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody variable region are according to the Kabat numbering system.

As used herein, the term “**affinity matured**” in the context of antigen binding molecules (e.g., antibodies) refers to an antigen binding molecule that is derived from a reference antigen binding molecule, e.g., by mutation, binds to the same antigen, preferably binds to the same epitope, as the reference antibody; and has a higher affinity for the antigen than that of the reference antigen binding molecule. Affinity maturation generally involves modification of one or more amino acid residues in one or more CDRs of the antigen binding molecule. Typically, the affinity matured antigen binding molecule binds to the same epitope as the initial reference antigen binding molecule.

“**Framework**” or “FR” refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

An “**acceptor human framework**” for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

The term “**chimeric**” antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

The “**class**” of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ respectively.

A “**humanized**” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody.

A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “**humanized form**” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization. Other forms of “humanized antibodies” encompassed by the present invention are those in which the constant region has
 5 been additionally modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding.

A “**human**” antibody is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source
 10 that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

The term “**CH1 domain**” denotes the part of an antibody heavy chain polypeptide that extends approximately from EU position 118 to EU position 215 (EU numbering system
 15 according to Kabat). In one aspect, a CH1 domain has the amino acid sequence of
 ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
 HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHNKPS NTKVDKKV (SEQ ID NO: 94).
 Usually, a segment having the amino acid sequence of EPKSC (SEQ ID NO:99) is following to link the CH1 domain to the hinge region,

20 The term “**hinge region**” denotes the part of an antibody heavy chain polypeptide that joins in a wild-type antibody heavy chain the CH1 domain and the CH2 domain, e. g. from about position 216 to about position 230 according to the EU number system of Kabat, or from about position 226 to about position 230 according to the EU number system of Kabat. The hinge regions of other IgG subclasses can be determined by aligning with the hinge-region cysteine
 25 residues of the IgG1 subclass sequence. The hinge region is normally a dimeric molecule consisting of two polypeptides with identical amino acid sequence. The hinge region generally comprises up to 25 amino acid residues and is flexible allowing the associated target binding sites to move independently. The hinge region can be subdivided into three domains: the upper, the middle, and the lower hinge domain (see e.g. Roux, et al., J. Immunol. 161 (1998) 4083).

30 In one aspect, the hinge region has the amino acid sequence DKTHTCPXCP (SEQ ID NO: 100), wherein X is either S or P. In one aspect, the hinge region has the amino acid sequence HTCPXCP (SEQ ID NO: 101), wherein X is either S or P. In one aspect, the hinge region has the amino acid sequence CPXCP (SEQ ID NO: 102), wherein X is either S or P.

The term “**Fc domain**” or “**Fc region**” herein is used to define a C-terminal region of an
 35 antibody heavy chain that contains at least a portion of the constant region. The term includes

native sequence Fc regions and variant Fc regions. In one aspect, a human IgG heavy chain Fc-domain extends from Cys226, or from Pro230, or from Ala231 to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc-region may or may not be present. An IgG Fc region comprises an IgG CH2 and an IgG CH3 domain.

5 The “CH2 domain” of a human IgG Fc region usually extends from an amino acid residue at about EU position 231 to an amino acid residue at about EU position 340 (EU numbering system according to Kabat). In one aspect, a CH2 domain has the amino acid sequence of
 APELLGGPSV FLFPPKPKDT LMISRTPEVT CVWDVSHEDP EVKFNWYVDG
 VEVHNAKTKP REEQUESTYRW SVLTVLHQDW LNKKEYKCKV SNKALPAPIE KTISKAK
 10 (SEQ ID NO: 95). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native Fc-region. It has been speculated that the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, Mol. Immunol. 22 (1985) 161-206. In one embodiment, a carbohydrate chain is attached to the CH2
 15 domain. The CH2 domain herein may be a native sequence CH2 domain or variant CH2 domain.

The “CH3 domain” comprises the stretch of residues C-terminal to a CH2 domain in an Fc region denotes the part of an antibody heavy chain polypeptide that extends approximately from EU position 341 to EU position 446 (EU numbering system according to Kabat). In one aspect, the CH3 domain has the amino acid sequence of GQPREPQVYT LPFSRDELTK NQVSLTCLVK
 20 GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG
 NVFSCSVME ALHNHYTQKS LSLSPG (SEQ ID NO: 96). The CH3 region herein may be a native sequence CH3 domain or a variant CH3 domain (e.g. a CH3 domain with an introduced “protuberance” (“knob”) in one chain thereof and a corresponding introduced “cavity” (“hole”) in the other chain thereof; see US Patent No. 5,821,333, expressly incorporated herein by
 25 reference). Such variant CH3 domains may be used to promote heterodimerization of two non-identical antibody heavy chains as herein described. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant
 30 region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

The “**knob-into-hole**” technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001).
 35 Generally, the method involves introducing a protuberance (“knob”) at the interface of a first polypeptide and a corresponding cavity (“hole”) in the interface of a second polypeptide, such

that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created
5 in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis. In a specific embodiment a knob modification comprises the amino acid substitution T366W in one of the two subunits of the Fc domain, and the hole modification comprises the amino acid
10 substitutions T366S, L368A and Y407V in the other one of the two subunits of the Fc domain. In a further specific embodiment, the subunit of the Fc domain comprising the knob modification additionally comprises the amino acid substitution S354C, and the subunit of the Fc domain comprising the hole modification additionally comprises the amino acid substitution Y349C. Introduction of these two cysteine residues results in the formation of a disulfide bridge between
15 the two subunits of the Fc region, thus further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)). The numbering is according to EU index of Kabat et al, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

A "region equivalent to the Fc region of an immunoglobulin" is intended to include
20 naturally occurring allelic variants of the Fc region of an immunoglobulin as well as variants having alterations which produce substitutions, additions, or deletions but which do not decrease substantially the ability of the immunoglobulin to mediate effector functions (such as antibody-dependent cellular cytotoxicity). For example, one or more amino acids can be deleted from the N-terminus or C-terminus of the Fc region of an immunoglobulin without substantial loss of
25 biological function. Such variants can be selected according to general rules known in the art so as to have minimal effect on activity (see, e.g., Bowie, J. U. et al., Science 247:1306-10 (1990)).

The term "**wild-type Fc domain**" denotes an amino acid sequence identical to the amino acid sequence of an Fc domain found in nature. Wild-type human Fc domains include a native human IgG1 Fc-region (non-A and A allotypes), native human IgG2 Fc-region, native human
30 IgG3 Fc-region, and native human IgG4 Fc-region as well as naturally occurring variants thereof. Wild-type Fc-regions are denoted in SEQ ID NO: 102 (IgG1, caucasian allotype), SEQ ID NO: 103 (IgG1, afroamerican allotype), SEQ ID NO: 104 (IgG2), SEQ ID NO: 105 (IgG3) and SEQ ID NO: 106 (IgG4).

The term "**variant (human) Fc domain**" denotes an amino acid sequence which differs
35 from that of a "wild-type" (human) Fc domain amino acid sequence by virtue of at least one "amino acid mutation". In one aspect, the variant Fc-region has at least one amino acid mutation

compared to a native Fc-region, e.g. from about one to about ten amino acid mutations, and in one aspect from about one to about five amino acid mutations in a native Fc-region. In one aspect, the (variant) Fc-region has at least about 95 % homology with a wild-type Fc-region.

The term “**effector functions**” refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

Fc receptor binding dependent effector functions can be mediated by the interaction of the Fc-region of an antibody with Fc receptors (FcRs), which are specialized cell surface receptors on hematopoietic cells. Fc receptors belong to the immunoglobulin superfamily, and have been shown to mediate both the removal of antibody-coated pathogens by phagocytosis of immune complexes, and the lysis of erythrocytes and various other cellular targets (e.g. tumor cells) coated with the corresponding antibody, via antibody dependent cell mediated cytotoxicity (ADCC) (see e.g. Van de Winkel, J.G. anderson, C.L., *J. Leukoc. Biol.* 49 (1991) 511-524). FcRs are defined by their specificity for immunoglobulin isotypes: Fc receptors for IgG antibodies are referred to as FcγR. Fc receptor binding is described e.g. in Ravetch, J.V. and Kinet, J.P., *Annu. Rev. Immunol.* 9 (1991) 457-492; Capel, P.J., et al., *Immunomethods* 4 (1994) 25-34; de Haas, M., et al., *J. Lab. Clin. Med.* 126 (1995) 330-341; and Gessner, J.E., et al., *Ann. Hematol.* 76 (1998) 231-248.

Cross-linking of receptors for the Fc-region of IgG antibodies (FcγR) triggers a wide variety of effector functions including phagocytosis, antibody-dependent cellular cytotoxicity, and release of inflammatory mediators, as well as immune complex clearance and regulation of antibody production. In humans, three classes of FcγR have been characterized, which are:

- FcγRI (CD64) binds monomeric IgG with high affinity and is expressed on macrophages, monocytes, neutrophils and eosinophils. Modification in the Fc-region IgG at least at one of the amino acid residues E233-G236, P238, D265, N297, A327 and P329 (numbering according to EU index of Kabat) reduce binding to FcγRI. IgG2 residues at positions 233–236, substituted into IgG1 and IgG4, reduced binding to FcγRI by 10³-fold and eliminated the human monocyte response to antibody-sensitized red blood cells (Armour, K.L., et al., *Eur. J. Immunol.* 29 (1999) 2613–2624).

- FcγRII (CD32) binds complexed IgG with medium to low affinity and is widely expressed. This receptor can be divided into two sub-types, FcγRIIA and FcγRIIB. FcγRIIA is found on many cells involved in killing (e.g. macrophages, monocytes, neutrophils) and seems

able to activate the killing process. FcγRIIB seems to play a role in inhibitory processes and is found on B cells, macrophages and on mast cells and eosinophils. On B-cells it seems to function to suppress further immunoglobulin production and isotype switching to, for example, the IgE class. On macrophages, FcγRIIB acts to inhibit phagocytosis as mediated through FcγRIIA. On eosinophils and mast cells the B-form may help to suppress activation of these cells through IgE binding to its separate receptor. Reduced binding for FcγRIIA is found e.g. for antibodies comprising an IgG Fc-region with mutations at least at one of the amino acid residues E233-G236, P238, D265, N297, A327, P329, D270, Q295, A327, R292, and K414 (numbering according to EU index of Kabat).

10 - FcγRIII (CD16) binds IgG with medium to low affinity and exists as two types. FcγRIIIA is found on NK cells, macrophages, eosinophils and some monocytes and T cells and mediates ADCC. Fc γ RIIB is highly expressed on neutrophils. Reduced binding to FcγRIIIA is found e.g. for antibodies comprising an IgG Fc-region with mutation at least at one of the amino acid residues E233-G236, P238, D265, N297, A327, P329, D270, Q295, A327, S239, E269, E293, 15 Y296, V303, A327, K338 and D376 (numbering according to EU index of Kabat).

Mapping of the binding sites on human IgG1 for Fc receptors, the above mentioned mutation sites and methods for measuring binding to FcγRI and FcγRIIA are described in Shields, R.L., et al. J. Biol. Chem. 276 (2001) 6591-6604.

20 The term “**ADCC**” or “antibody-dependent cellular cytotoxicity” is a function mediated by Fc receptor binding and refers to lysis of target cells by an antibody as reported herein in the presence of effector cells. The capacity of the antibody to induce the initial steps mediating ADCC is investigated by measuring their binding to Fcγ receptors expressing cells, such as cells, recombinantly expressing FcγRI and/or FcγRIIA or NK cells (expressing essentially FcγRIIIA). In particular, binding to FcγR on NK cells is measured.

25 An “**activating Fc receptor**” is an Fc receptor that following engagement by an Fc region of an antibody elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Activating Fc receptors include FcγRIIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32), and FcαRI (CD89). A particular activating Fc receptor is human FcγRIIIa (see UniProt accession no. P08637, version 141).

30 The “**Tumor Necrosis factor receptor superfamily**” or “**TNF receptor superfamily**” currently consists of 27 receptors. It is a group of cytokine receptors characterized by the ability to bind tumor necrosis factors (TNFs) via an extracellular cysteine-rich domain (CRD). These pseudorepeats are defined by intrachain disulphides generated by highly conserved cysteine residues within the receptor chains. With the exception of nerve growth factor (NGF), all TNFs 35 are homologous to the archetypal TNF-alpha. In their active form, the majority of TNF receptors form trimeric complexes in the plasma membrane. Accordingly, most TNF receptors contain

transmembrane domains (TMDs). Several of these receptors also contain intracellular death domains (DDs) that recruit caspase-interacting proteins following ligand binding to initiate the extrinsic pathway of caspase activation. Other TNF superfamily receptors that lack death domains bind TNF receptor-associated factors and activate intracellular signaling pathways that can lead to proliferation or differentiation. These receptors can also initiate apoptosis, but they do so via indirect mechanisms. In addition to regulating apoptosis, several TNF superfamily receptors are involved in regulating immune cell functions such as B cell homeostasis and activation, natural killer cell activation, and T cell co-stimulation. Several others regulate cell type-specific responses such as hair follicle development and osteoclast development. Members of the TNF receptor superfamily include the following: Tumor necrosis factor receptor 1 (1A) (TNFRSF1A, CD120a), Tumor necrosis factor receptor 2 (1B) (TNFRSF1B, CD120b), Lymphotoxin beta receptor (LTBR, CD18), OX40 (TNFRSF4, CD134), CD40 (Bp50), Fas receptor (Apo-1, CD95, FAS), Decoy receptor 3 (TR6, M68, TNFRSF6B), CD27 (S152, Tp55), CD30 (Ki-1, TNFRSF8), 4-1BB (CD137, TNFRSF9), DR4 (TRAILR1, Apo-2, CD261, TNFRSF10A), DR5 (TRAILR2, CD262, TNFRSF10B), Decoy Receptor 1 (TRAILR3, CD263, TNFRSF10C), Decoy Receptor 2 (TRAILR4, CD264, TNFRSF10D), RANK (CD265, TNFRSF11A), Osteoprotegerin (OCIF, TR1, TNFRSF11B), TWEAK receptor (Fn14, CD266, TNFRSF12A), TACI (CD267, TNFRSF13B), BAFF receptor (CD268, TNFRSF13C), Herpesvirus entry mediator (HVEM, TR2, CD270, TNFRSF14), Nerve growth factor receptor (p75NTR, CD271, NGFR), B-cell maturation antigen (CD269, TNFRSF17), Glucocorticoid-induced TNFR-related (GITR, AITR, CD357, TNFRSF18), TROY (TNFRSF19), DR6 (CD358, TNFRSF21), DR3 (Apo-3, TRAMP, WS-1, TNFRSF25) and Ectodysplasin A2 receptor (XEDAR, EDA2R).

Several members of the tumor necrosis factor receptor (TNFR) family function after initial T cell activation to sustain T cell responses. The term “**costimulatory TNF receptor family member**” or “costimulatory TNF family receptor” refers to a subgroup of TNF receptor family members, which are able to costimulate proliferation and cytokine production of T-cells. The term refers to any native TNF family receptor from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. In specific embodiments of the invention, costimulatory TNF receptor family members are selected from the group consisting of OX40 (CD134), 4-1BB (CD137), CD40, CD27, HVEM (CD270), CD30, and GITR, all of which can have costimulatory effects on T cells. More particularly, the antigen binding molecule of the present invention comprises at least moiety capable of specific binding to the costimulatory TNF receptor family member OX40.

Further information, in particular sequences, of the TNF receptor family members may be obtained from publically accessible databases such as Uniprot (www.uniprot.org). For instance,

the human costimulatory TNF receptors have the following amino acid sequences: human OX40 (UniProt accession no. P43489, SEQ ID NO:108), human 4-1BB (UniProt accession no. Q07011, SEQ ID NO:109), human CD27 (UniProt accession no. P26842, SEQ ID NO:110), human HVEM (UniProt accession no. Q92956, SEQ ID NO:111), human CD30 (UniProt
5 accession no. P28908, SEQ ID NO:112), human GITR (UniProt accession no. Q9Y5U5, SEQ ID NO:113) and human CD40 (UniProt accession no. P25942, SEQ ID NO:115).

The term “**OX40**”, as used herein, refers to any native OX40 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed OX40 as well as any form
10 of OX40 that results from processing in the cell. The term also encompasses naturally occurring variants of OX40, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human OX40 is shown in SEQ ID NO: 107 (Uniprot P43489, version 112) and the amino acid sequence of an exemplary murine OX40 is shown in SEQ ID NO: 113 (Uniprot P47741, version 101).

15 Among several costimulatory molecules, the tumor necrosis factor (TNF) receptor family member OX40 (CD134) plays a key role in the survival and homeostasis of effector and memory T cells (Croft M. et al. (2009), Immunological Reviews 229, 173-191). OX40 (CD134) is expressed in several types of cells and regulates immune responses against infections, tumors and self-antigens and its expression has been demonstrated on the surface of T-cells, NKT-cells
20 and NK-cells as well as neutrophils (Baumann R. et al. (2004), Eur. J. Immunol. 34, 2268-2275) and shown to be strictly inducible or strongly upregulated in response to various stimulatory signals. Functional activity of the molecule has been demonstrated in every OX40-expressing cell type suggesting complex regulation of OX40-mediated activity *in vivo*. Combined with T-cell receptor triggering, OX40 engagement on T-cells by its natural ligand or agonistic
25 antibodies leads to synergistic activation of the PI3K and NFκB signalling pathways (Song J. et al. (2008) J. Immunology 180(11), 7240-7248). In turn, this results in enhanced proliferation, increased cytokine receptor and cytokine production and better survival of activated T-cells. In addition to its co-stimulatory activity in effector CD4⁺ or CD8⁺ T-cells, OX40 triggering has been recently shown to inhibit the development and immunosuppressive function of T regulatory
30 cells. This effect is likely to be responsible, at least in part, for the enhancing activity of OX40 on anti-tumor or anti-microbial immune responses. Given that OX40 engagement can expand T-cell populations, promote cytokine secretion, and support T-cell memory, agonists including antibodies and soluble forms of the ligand OX40L have been used successfully in a variety of preclinical tumor models (Weinberg et al. (2000), J. Immunol. 164, 2160–2169).

35 The terms “**anti-OX40 antibody**”, “anti-OX40”, “OX40 antibody and “an antibody that specifically binds to OX40” refer to an antibody that is capable of binding OX40 with sufficient

affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting OX40. In one embodiment, the extent of binding of an anti-OX40 antibody to an unrelated, non-OX40 protein is less than about 10% of the binding of the antibody to OX40 as measured, e.g., by flow cytometry (FACS). In certain embodiments, an antibody that binds to OX40 has a dissociation constant (K_D) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (e.g. 10^{-6} M or less, e.g. from 10^{-68} M to 10^{-13} M , e.g., from 10^{-8} M to 10^{-10} M).

The term “**4-1BB**” or “**CD137**”, as used herein, refers to any native 4-1BB from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed 4-1BB as well as any form of 4-1BB that results from processing in the cell. The term also encompasses naturally occurring variants of 4-1BB, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human 4-1BB is shown in SEQ ID NO:109 (Uniprot accession no. Q07011).

The terms “**anti-4-1BB antibody**”, “anti-4-1BB”, “4-1BB antibody and “an antibody that specifically binds to 4-1BB” refer to an antibody that is capable of binding 4-1BB with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting 4-1BB. In one embodiment, the extent of binding of an anti-4-1BB antibody to an unrelated, non-4-1BB protein is less than about 10% of the binding of the antibody to 4-1BB as measured, e.g., by a radioimmunoassay (RIA) or flow cytometry (FACS). In certain embodiments, an antibody that binds to 4-1BB has a dissociation constant (K_D) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (e.g. 10^{-6} M or less, e.g. from 10^{-68} M to 10^{-13} M , e.g., from 10^{-8} M to 10^{-10} M). In particular, the anti-4-1BB antibody is clone 20H4.9 as disclosed in US Patent No. 7,288,638.

The term “**CD40**”, as used herein, refers to any native CD40 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed CD40 as well as any form of CD40 that results from processing in the cell. The term also encompasses naturally occurring variants of CD40, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human CD40 is shown in SEQ ID NO:115 (UniProt no. P25942, version 200). The CD40 antigen is a 50 kDa cell surface glycoprotein which belongs to the Tumor Necrosis Factor Receptor (TNF-R) family. (Stamenkovic et al. (1989), EMBO J. 8: 1403-10). CD40 is expressed in many normal and tumor cell types, including B lymphocytes, dendritic cells, monocytes, macrophages, thymus epithelium, endothelial cells, fibroblasts, and smooth muscle cells. CD40 is expressed in all B-lymphomas and in 70% of all solid tumors and is up-regulated in antigen presenting cells (APCs) by maturation signals, such as IFN-gamma and GM-CSF. CD40 activation also induces differentiation of monocytes into functional dendritic cells (DCs) and

enhances cytolytic activity of NK cells through APC-CD40 induced cytokines. Thus CD40 plays an essential role in the initiation and enhancement of immune responses by inducing maturation of APCs, secretion of helper cytokines, upregulation of costimulatory molecules, and enhancement of effector functions.

5 The term "**CD40 agonist**" as used herein includes any moiety that agonizes the CD40/CD40L interaction. CD40 as used in this context refers preferably to human CD40, thus the CD40 agonist is preferably an agonist of human CD40. Typically, the moiety will be an agonistic CD40 antibody or antibody fragment.

10 The terms "**anti-CD40 antibody**", "anti-CD40", "CD40 antibody" and "an antibody that specifically binds to CD40" refer to an antibody that is capable of binding CD40 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD40. In one aspect, the extent of binding of an anti-CD40 antibody to an unrelated, non-CD40 protein is less than about 10% of the binding of the antibody to CD40 as measured, e.g., by a radioimmunoassay (RIA) or flow cytometry (FACS). In certain embodiments, an antibody that
15 binds to CD40 has a dissociation constant (K_D) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (e.g. 10^{-6} M or less, e.g. from 10^{-68} M to 10^{-13} M , e.g., from 10^{-8} M to 10^{-10} M).

20 The term "**peptide linker**" refers to a peptide comprising one or more amino acids, typically about 2 to 20 amino acids. Peptide linkers are known in the art or are described herein. Suitable, non-immunogenic linker peptides are, for example, $(G_4S)_n$, $(SG_4)_n$ or $G_4(SG_4)_n$ peptide linkers, wherein "n" is generally a number between 1 and 10, typically between 1 and 4, in particular 2, i.e. the peptides selected from the group consisting of GGGGS (SEQ ID NO:77), GGGGSGGGGS (SEQ ID NO:78), SGGGSGGGG (SEQ ID NO:79), GGGGSGGGGSSGGGS (SEQ ID NO:80), $(G_4S)_3$ or GGGGSGGGGSGGGGS (SEQ ID
25 NO:81), GGGGSGGGGSGGGG or $G_4(SG_4)_2$ (SEQ ID NO:82), $(G_4S)_4$ or GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:83), and GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:84), but also include the sequences GSPGSSSSGS (SEQ ID NO:85), GSGSGSGS (SEQ ID NO:86), GSGSGNGS (SEQ ID NO:87), GGSGSGSG (SEQ ID NO:88), GGSGSG (SEQ ID NO:89), GGSG (SEQ ID NO:90), GGSGNGSG (SEQ ID NO:91), GGNGSGSG (SEQ ID
30 NO:92) and GGNGSG (SEQ ID NO:93). Peptide linkers of particular interest are $((G_4S)_2$ or GGGGSGGGGS (SEQ ID NO:78) and GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:84).

35 A "**spacer domain**" according to the present invention is a polypeptide forming a structural domain after folding. Thus, the spacer domain can be smaller than 100 amino acid residues, but needs to be structurally confined to fix the binding motifs. Exemplary spacer domains are pentameric coil-coils, antibody hinge regions or antibody Fc regions or fragments

thereof. The spacer domain is a dimerization domain, i.e. the the spacer domain comprises amino acids that are able to provide the dimerization functionality.

The term "**amino acid**" as used within this application denotes the group of naturally occurring carboxy α -amino acids comprising alanine (three letter code: ala, one letter code: A),
5 arginine (arg, R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V).

By "**fused**" or "**connected**" is meant that the components (e.g. a heavy chain of an
10 antibody and a Fab fragment) are linked by peptide bonds, either directly or via one or more peptide linkers.

A "**fusion polypeptide**" or "single fusion polypeptide" as used herein refers to a single chain polypeptide composed of different components such as the ectodomain of a TNF ligand family member that are fused to each either directly or via a peptide linker. By "fused" or
15 "connected" is meant that the components (e.g. a polypeptide and an ectodomain of said TNF ligand family member) are linked by peptide bonds, either directly or via one or more peptide linkers.

"**Percent (%) amino acid sequence identity**" with respect to a reference polypeptide (protein) sequence is defined as the percentage of amino acid residues in a candidate sequence
20 that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available
25 computer software such as BLAST, BLAST-2, ALIGN, SAWI or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-
30 2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX
35 operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by

the ALIGN-2 program and do not vary. In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

In certain embodiments, **amino acid sequence variants** of the antigen binding molecules provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antigen binding molecules. Amino acid sequence variants of the antigen binding molecules may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the molecules, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding. Sites of interest for substitutional mutagenesis include the HVRs and Framework (FRs). Conservative substitutions are provided in Table B under the heading "Preferred Substitutions" and further described below in reference to amino acid side chain classes (1) to (6). Amino acid substitutions may be introduced into the molecule of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE A

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser

Original Residue	Exemplary Substitutions	Preferred Substitutions
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- 5 (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

10 Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

The term “**amino acid sequence variants**” includes substantial variants wherein there are amino acid substitutions in one or more hypervariable region residues of a parent antigen binding molecule (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antigen binding molecule and/or will have substantially retained certain biological properties of the parent antigen binding molecule. An exemplary substitutional variant is an affinity matured

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antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antigen binding molecules displayed on phage and screened for a particular biological activity (e.g. binding affinity). In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antigen binding molecule to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antigen binding molecule complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include bispecific antigen binding molecules of the invention with an N-terminal methionyl residue. Other insertional variants of the molecule include the fusion to the N- or C-terminus to a polypeptide which increases the serum half-life of the bispecific antigen binding molecules.

In certain embodiments, the bispecific antigen binding molecules provided herein are altered to increase or decrease the extent to which the antibody is glycosylated. **Glycosylation variants** of the molecules may be conveniently obtained by altering the amino acid sequence such that one or more glycosylation sites is created or removed. Where the bispecific antigen binding molecule comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in the antigen binding

molecule may be made in order to create variants with certain improved properties. In one aspect, variants of bispecific antigen binding molecules or antibodies of the invention are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. Such fucosylation variants may have improved ADCC function, see e.g. US Patent
5 Publication Nos. US 2003/0157108 (Presta, L.) or US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). In another aspect, variants of the bispecific antigen binding molecules or antibodies of the invention are provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region is bisected by GlcNAc. Such variants may have reduced fucosylation and/or improved ADCC function., see for example WO 2003/011878
10 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function and are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

15 In certain aspects, it may be desirable to create **cysteine engineered variants** of the bispecific antigen binding molecules of the invention, e.g., “thioMAbs,” in which one or more residues of the molecule are substituted with cysteine residues. In particular aspects, the substituted residues occur at accessible sites of the molecule. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may
20 be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate. In certain aspects, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antigen binding molecules may be generated as described, e.g., in U.S.
25 Patent No. 7,521,541.

In certain aspects, the bispecific antibody provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to,
30 to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers,
35 polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of

polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the bispecific antibody derivative will be used in a therapy under defined conditions, etc. In another aspect, conjugates of an antibody and non-proteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the non-proteinaceous moiety is a carbon nanotube (Kam, N.W. et al., Proc. Natl. Acad. Sci. USA 102 (2005) 11600-11605). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the non-proteinaceous moiety to a temperature at which cells proximal to the antibody-non-proteinaceous moiety are killed.

In another aspect, immunoconjugates of the bispecific antibodies provided herein may be obtained. An **"immunoconjugate"** is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

The term **"nucleic acid"** refers to an isolated nucleic acid molecule or construct, e.g. messenger RNA (mRNA), virally-derived RNA, or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (e.g. an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid molecule" refers to any one or more nucleic acid segments, e.g. DNA or RNA fragments, present in a polynucleotide.

By **"isolated"** nucleic acid molecule or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. An isolated polynucleotide includes a polynucleotide molecule contained in cells that ordinarily contain the polynucleotide molecule, but the polynucleotide molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the present invention, as well as positive and negative strand forms, and double-stranded forms. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the

nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides
5 in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or
10 more contiguous groups within the reference sequence. As a practical matter, whether any particular polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs, such as the ones discussed above for polypeptides (e.g. ALIGN-2).

15 The term "**expression cassette**" refers to a polynucleotide generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among
20 other sequences, a nucleic acid sequence to be transcribed and a promoter. In certain embodiments, the expression cassette of the invention comprises polynucleotide sequences that encode bispecific antigen binding molecules of the invention or fragments thereof.

The term "**vector**" or "expression vector" is synonymous with "expression construct" and refers to a DNA molecule that is used to introduce and direct the expression of a specific gene to
25 which it is operably associated in a target cell. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. The expression vector of the present invention comprises an expression cassette. Expression vectors allow transcription of large amounts of stable mRNA. Once the expression vector is inside the target cell, the ribonucleic acid molecule or protein that is
30 encoded by the gene is produced by the cellular transcription and/or translation machinery. In one embodiment, the expression vector of the invention comprises an expression cassette that comprises polynucleotide sequences that encode bispecific antigen binding molecules of the invention or fragments thereof.

The terms "**host cell**", "host cell line," and "host cell culture" are used interchangeably and
35 refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary

transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. A host cell is any type of
5 cellular system that can be used to generate the bispecific antigen binding molecules of the present invention. Host cells include cultured cells, e.g. mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or
10 animal tissue.

An "**effective amount**" of an agent refers to the amount that is necessary to result in a physiological change in the cell or tissue to which it is administered.

A "**therapeutically effective amount**" of an agent, e.g. a pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired
15 therapeutic or prophylactic result. A therapeutically effective amount of an agent for example eliminates, decreases, delays, minimizes or prevents adverse effects of a disease.

An "**individual**" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g. cows, sheep, cats, dogs, and horses), primates (e.g. humans and non-human primates such as monkeys), rabbits, and rodents (e.g. mice and rats). Particularly, the
20 individual or subject is a human.

The term "**pharmaceutical composition**" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

25 A "**pharmaceutically acceptable excipient**" refers to an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable excipient includes, but is not limited to, a buffer, a stabilizer, or a preservative.

The term "**package insert**" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications,
30 usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

As used herein, "**treatment**" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual

being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease
5 progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, the molecules of the invention are used to delay development of a disease or to slow the progression of a disease.

The term “**cancer**” as used herein refers to proliferative diseases, such as lymphomas, carcinoma, lymphoma, blastoma, sarcoma, leukemia, lymphocytic leukemias, lung cancer, non-
10 small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colorectal cancer (CRC), pancreatic cancer, breast cancer, triple-negative breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix,
15 carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary
20 cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma and Ewings sarcoma, melanoma, multiple myeloma, B-cell cancer (lymphoma), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), hairy cell leukemia, chronic myeloblastic leukemia, including
25 refractory versions of any of the above cancers, or a combination of one or more of the above cancers..

Bispecific antigen binding molecules of the invention

The invention provides novel bispecific antibodies with particularly advantageous properties such as producibility, stability, binding affinity, biological activity, targeting
30 efficiency and reduced toxicity. The novel bispecific antibodies consist of two fusion polypeptides comprising two antigen binding domains capable of specific binding to a first target and one antigen binding domain capable of specific binding to a second target. Surprisingly, these two fusion polypeptides are engineered in a way that the three antigen binding domains can assemble correctly and that the bispecific binding is fully functional.

For a molecule intended to be developed towards clinical application, aggregates of functionally active molecules have to be avoided, meaning the purity and stability of the assembly of the different fusion polypeptides forming the antigen binding molecule is very critical. Importantly, in the bispecific antibodies of the invention, all three antigen binding domains are fused in a way that enables the correct assembly of the three antigen binding domains. And all three antigen binding domains are positioned in a way that every antigen binding domain can bind to their respective targets. The new bispecific antigen binding molecules of the invention are furthermore comprised only of two fusion polypeptides and do not comprise any light chains. Thus, the problem of correct pairing between heavy and light chains can be avoided. Important is also that the constructs are expressible with reasonably good titers and produce a good ratio of the wished product. The antibody-like architecture comprising a spacer domain for dimerization is stable compared to other proteins; their expression is also very robust using different cell lines.

The novel bispecific antigen binding molecules of the invention are called 2+1 Contorsbodies.

Thus, a 2+1 Contorsbody is a bispecific antibody consisting of two fusion polypeptides and comprising two antigen binding domains capable of specific binding to a first target and one antigen binding domain capable of specific binding to a second target, wherein

- (a) the first fusion polypeptide comprises a first part of a first antigen binding domain capable of specific binding to the first target, a spacer domain, a second part of a first antigen binding domain capable of specific binding to the first target and a first part of an antigen binding domain capable of specific binding to the second target, wherein
- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
 - the first part of the first antigen binding domain capable of specific binding to the first target is fused either directly or via a first peptide linker to the N-terminus of the spacer domain,
 - the second part of the first antigen binding domain capable of specific binding to the first target is fused either directly or via a second peptide linker to the C-terminus of the spacer domain, and
 - the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the first antigen binding domain capable of specific binding to the first target or is fused either directly or via a third peptide linker to the N-terminus of the

first part of the first antigen binding domain capable of specific binding to the first target, and

(b) the second fusion polypeptide comprising a first part of a second antigen binding domain capable of specific binding to a first target, a spacer domain, a second part of the second antigen binding domain capable of specific binding to a first target and the second part of an antigen binding domain capable of specific binding to a second target, wherein

- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,

- the first part of the second antigen binding domain capable of specific binding to a first target is fused either directly or via a first peptide linker to the N-terminus of the spacer domain,

- the second part of the second antigen binding domain capable of specific binding to a first target is fused either directly or via a second peptide linker to the C-terminus of the spacer domain, and

- the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the second antigen binding domain capable of specific binding to a first target or is fused either directly or via a third peptide linker to the N-terminus of the first part of the second antigen binding domain capable of specific binding to a first target,

wherein the first part and the second part of the antigen binding domain capable of specific binding to the second target are associated with each other to form the antigen binding domain capable of specific binding to the second target and wherein the first part and the second part of the first and second antigen binding domain capable of specific binding to the first target are associated with each other to form a circular fusion polypeptide, and

wherein the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide are associated covalently to each other by a disulfide bond and comprise modifications promoting the association of the first and second fusion polypeptide.

In one aspect, provided is a bispecific antibody as defined herein before, wherein in the first fusion polypeptide the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the first antigen binding domain capable of specific binding to the first target and wherein in the second fusion polypeptide the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-

terminus of the second part of the first antigen binding domain capable of specific binding to the first target.

In another aspect, provided is a bispecific antibody as defined herein before, wherein in the first fusion polypeptide the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the N-terminus of the first part of the first antigen binding domain capable of specific binding to the first target and wherein in the second fusion polypeptide the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the N-terminus of the first part of the first antigen binding domain capable of specific binding to the first target.

Thus, in the bispecific antibody as defined herein before, the original antibody domains are fused by flexible peptide linkers. These linkers enable the correct domain association within the Contorsbody molecule, as well as proper folding of the antibody. This new chain topology results in a spatial orientation of Fab arms and Fc part that differs from the classical IgG1 format. Due to the parallel orientation of its antigen binding sites, the Contorsbody is a very suitable antibody format for agonistic mechanisms.

In one aspect, provided is a bispecific antibody as defined herein before, wherein the third peptide linker connecting the first part or the second part of an antigen binding domain capable of specific binding to a second target comprises at least 15 amino acids. In one aspect, the third peptide linker connecting the first part of an antigen binding domain capable of specific binding to a second target and the third peptide linker connecting the second part of an antigen binding domain capable of specific binding to a second target are identical. In one aspect, the third peptide linker comprises 15 to 25 amino acids. In one particular aspect, the third peptide linker comprises the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:84. More particularly, the third peptide linker comprises the amino acid sequence of SEQ ID NO:84. In a further aspect, the third peptide linker (in both fusion polypeptides) comprises the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:84 and the first and second peptide linker comprises the amino acid sequence of SEQ ID NO:78.

In one aspect, the invention provides a bispecific antibody as defined herein before, wherein the first fusion polypeptide comprises the heavy chain variable domain of the antigen binding domain capable of specific binding to a second target and the second fusion polypeptide comprises the antibody light chain variable domain of the antigen binding domain capable of specific binding to a second target or vice versa.

In one aspect, the invention provides a bispecific antibody as defined herein before, wherein the first fusion polypeptide comprises the heavy chain variable domain of the antigen

binding domain capable of specific binding to a second target and the second fusion polypeptide comprises the antibody light chain variable domain of the antigen binding domain capable of specific binding to a second target or vice versa. In one particular aspect, the first part of the antigen binding domain is an antibody heavy chain Fab fragment and the second part of the antigen binding domain is an antibody light chain Fab fragment or vice versa. In one aspect, the first part of the antigen binding domain and the second part of the antigen binding domain are associated covalently to each other by a disulfide bond.

- In an alternative aspect, a bispecific antibody consisting of two fusion polypeptides and comprising two antigen binding domains capable of specific binding to a first target and one antigen binding domain capable of specific binding to a second target, wherein
- (a) the first fusion polypeptide comprises a first part of a first antigen binding domain capable of specific binding to the first target, a spacer domain, a second part of a first antigen binding domain capable of specific binding to the first target and a first part of an antigen binding domain capable of specific binding to the second target, wherein
- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
 - the first part of the first antigen binding domain capable of specific binding to the first target is fused either directly or via a first peptide linker to the N-terminus of the spacer domain,
 - the second part of the first antigen binding domain capable of specific binding to the first target is fused either directly or via a second peptide linker to the C-terminus of the spacer domain, and
 - the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the first antigen binding domain capable of specific binding to the first target or is fused either directly or via a third peptide linker to the N-terminus of the first part of the first antigen binding domain capable of specific binding to the first target, and
- (b) the second fusion polypeptide comprising a first part of a second antigen binding domain capable of specific binding to a first target, a spacer domain, a second part of the second antigen binding domain capable of specific binding to a first target and the second part of an antigen binding domain capable of specific binding to a second target, wherein
- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,

- the first part of the second antigen binding domain capable of specific binding to a first target is fused either directly or via a first peptide linker to the N-terminus of the spacer domain,

5 - the second part of the second antigen binding domain capable of specific binding to a first target is fused either directly or via a second peptide linker to the C-terminus of the spacer domain, and

(c) a light chain comprising the the second part of an antigen binding domain capable of specific binding to a second target,

10 wherein the first part and the second part of the antigen binding domain capable of specific binding to the second target are associated with each other to form the antigen binding domain capable of specific binding to the second target and wherein the first part and the second part of the first and second antigen binding domain capable of specific binding to the first target are associated with each other to form a circular fusion polypeptide, and

15 wherein the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide are associated covalently to each other by a disulfide bond and comprise modifications promoting the association of the first and second fusion polypeptide.

20 In some aspects, the first part of the antigen binding domain is an antibody heavy chain Fab fragment and the second part of the antigen binding domain is an antibody light chain Fab fragment or vice versa. In one aspect, the first part of the antigen binding domain and the second part of the antigen binding domain are associated covalently to each other by a disulfide bond.

25 In one aspect, provided is a bispecific antibody as defined herein before, wherein in both the first fusion polypeptide and the second fusion polypeptide the first part of the antigen binding domain capable of specific binding to the first target is an antibody heavy chain Fab fragment and the second part of the antigen binding domain capable of specific binding to the first target is an antibody light chain Fab fragment.

 If the antigen binding domain is a Fab fragment, then the Fab can be a conventional Fab, a cross-Fab or a DutaFab.

30 In case of a conventional Fab, a first part of the antigen binding domain comprises an antibody heavy chain variable domain (VH) and at least an N-terminal fragment of a (or a complete) first antibody heavy chain constant domain (CH1) and the respective second part of the antigen binding domain comprises an antibody light chain variable domain (VL) and at least an N-terminal fragment of a (or a complete) antibody light chain constant domain (CL or Ckappa). The order of these domains may be any as long as association thereof and forming of a

(functional) antigen binding domain is possible (i.e. not prevented). In one aspect, one part of the antigen binding domain comprises in N- to C-terminal direction VH-CH1 and the other part of the antigen binding domain comprises in N- to C-terminal direction VL-CL (Ckappa).

5 In case of a cross-Fab both parts of the antigen binding domain comprise each an antibody variable domain and at least an N-terminal fragment of a (or a complete) antibody constant domain whereby the pairs of variable domain and constant domain are not naturally associated with each other and are obtained by a domain cross-over/exchange of a heavy chain domain and a light chain domain. This can be the exchange of VH with VL or CH1 with CL. The order of these domains may be any as long as association thereof and forming of a (functional) binding
10 site is possible (i.e. not prevented). In one aspect, the first part of the antigen binding domain comprises in N- to C-terminal direction VL-CH1 and the second part of the binding domain comprises in N- to C-terminal direction VH-CL (Ckappa). In another aspect, the first part of the antigen binding domain comprises in N- to C-terminal direction VH-CL and the second part of the binding domain comprises in N- to C-terminal direction VL-CH1.

15 In case of a DutaFab, a first part of the antigen binding domain comprises an antibody heavy chain variable domain (VH) and at least an N-terminal fragment of a (or a complete) first antibody heavy chain constant domain (CH1) and the respective second antigen binding domain comprises an antibody light chain variable domain (VL) and at least an N-terminal fragment of a (or a complete) antibody light chain constant domain (CL), wherein said antigen binding domain
20 comprises two non-overlapping paratopes in the complementary pair of a heavy chain variable domain (VH) and a light chain variable domain (VL), wherein the first paratope comprises residues from CDR1 and CDR3 of the VL domain and CDR2 of the VH domain, and the second paratope comprises residues from CDR1 and CDR3 of the VH domain and CDR2 of the VL domain.

25 Thus, in one aspect, the first part of the antigen binding domain is an antibody heavy chain Fab fragment (VH-CH1) and the second part of the antigen binding domain is an antibody light chain Fab fragment (VL-Ckappa). In another aspect, the first part of the antigen binding domain is an antibody light chain Fab fragment and the second part of the antigen binding domain is an antibody heavy chain Fab fragment. In another aspect, the first part of the antigen binding
30 domain is an antibody cross Fab fragment comprising VH-Ckappa and the second part of the antigen binding domain is an antibody cross Fab fragment comprising VL-CH1. In a further aspect, the first part of the antigen binding domain is an antibody cross Fab fragment comprising VL-CH1 and the second part of the antigen binding domain is an antibody cross Fab fragment comprising VH-Ckappa.

In a particular aspect, the invention provides a bispecific antibody, wherein the antigen binding domain capable of specific binding to a second target is a cross-Fab and wherein both antigen binding domains capable of specific binding to the first target are conventional Fabs.

5 In another aspect, the invention provides a bispecific antibody, wherein the antigen binding domain capable of specific binding to a second target is a conventional Fab and wherein both antigen binding domains capable of specific binding to the first target are cross-Fabs.

As described above, the bispecific antibody consists of a first and a second fusion polypeptide, both comprising a spacer domain, the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide are associated covalently to each other
10 by a disulfide bond and comprise modifications promoting the association of the first and second fusion polypeptide. The spacer domain comprises at least 25 amino acids.

In one aspect of the invention, the spacer domain comprises an antibody hinge region or a (C-terminal) fragment thereof and an antibody CH2 domain or a (N-terminal) fragment thereof. In another aspect, the spacer domain comprises an antibody hinge region or a fragment thereof,
15 an antibody CH2 domain, and an antibody CH3 domain or a fragment thereof. In one aspect, the spacer domain of the fusion polypeptide as described herein is an antibody Fc domain, in particular of IgG1, IgG2 or IgG4 subclass, more particularly of IgG1 subclass.

In one aspect, the spacer domain comprises a Fc domain with an amino acid sequence selected from the group consisting of SEQ ID NO:103, Seq ID NO:104, SEQ ID NO:105, SEQ
20 ID NO:106 and SEQ ID NO:107, or a variant of 95% homology thereof.

Furthermore, the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide comprise modifications promoting the association of the first and second fusion polypeptide. In a particular aspect, the spacer domain of the first fusion polypeptide comprises holes and the spacer domain of the second fusion polypeptide comprises
25 knobs according to the knobs into hole method. In a further aspect, the invention comprises a bispecific antibody, wherein the spacer domain comprises an antibody hinge region or a fragment thereof and an IgG1 Fc domain. Particularly, the IgG1 Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcγ receptor. In a particular aspect, the IgG1 Fc domain comprises the amino acid substitutions
30 L234A and L235A. In another aspect, the IgG1 Fc domain comprises the mutation P329G. More particularly, the IgG1 Fc domain comprises the amino acid substitutions L234A, L235A and P329G (numbering according to Kabat EU index).

In another aspect, the Fc domain as reported herein is of IgG1 or IgG2 subclass and comprises the mutations PVA236, GLPSS331, and/or L234A/L235A/P329G (numbering

according to Kabat EU index). In a further aspect, the Fc domain reported herein is of IgG4 subclass and comprises the mutation L235E. In one aspect, the Fc domain further comprises the mutation S228P. In one aspect, the Fc domain of IgG4 subclass comprises the mutation P329G. In one aspect, the Fc domain as reported herein is of IgG4 subclass and comprises the mutations
5 S228P/L235E/P329G (all numbering according to EU index of Kabat).

In some aspects, provided is a bispecific antibody wherein the one antigen binding domain capable of specific binding to a second target is an antigen binding domain capable of specific binding to a tumor associated antigen (TAA). In particular, the tumor associated antigen is Fibroblast Activation Protein (FAP). In one aspect, provided is a bispecific antibody, wherein the
10 antigen binding domain capable of specific binding to a second target is an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP).

Fc domain modifications promoting heterodimerization

In one aspect, the bispecific antibodies of the invention may comprise (a) a first fusion polypeptide as defined herein before and a second fusion polypeptide as defined herein before,
15 wherein the first and second fusion polypeptide comprise modifications promoting the association of the first and second fusion polypeptide. Typically, these modifications are introduced in the Fc domains. Recombinant co-expression of the two structurally different fusion polypeptides and subsequent dimerization would lead to several possible combinations of the two polypeptides. In order to improve the yield and purity of the bispecific antibodies in
20 recombinant production, it will thus be advantageous to introduce in the Fc domain of the bispecific antigen binding molecules of the invention modifications promoting the association of the desired polypeptides.

The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain of the Fc domain. Thus, said modification is particularly in
25 the CH3 domain of the Fc domain.

In a specific aspect, said modification is a so-called “knob-into-hole” modification, comprising a “knob” modification in one of the two subunits of the Fc domain and a “hole” modification in the other one of the two subunits of the Fc domain. Thus, in a particular aspect, the invention relates to a bispecific antigen binding molecule as described herein before which
30 comprises an IgG molecule, wherein the Fc part of the first heavy chain comprises a first dimerization module and the Fc part of the second heavy chain comprises a second dimerization module allowing a heterodimerization of the two heavy chains of the IgG molecule and the first dimerization module comprises knobs and the second dimerization module comprises holes according to the knob into hole technology.

The knob-into-hole technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., *Prot Eng* 9, 617-621 (1996) and Carter, *J Immunol Meth* 248, 7-15 (2001). Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the

5 protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones

10 (e.g. alanine or threonine).

The CH3 domains in the first and second fusion polypeptide as reported herein can be altered by the "knob-into-holes" technology which is described in detail with several examples in e.g. WO 96/027011, Ridgway, J.B., et al., *Protein Eng.* 9 (1996) 617-621; and Merchant, A.M., et al., *Nat. Biotechnol.* 16 (1998) 677-681. In this method the interaction surfaces of the two

15 CH3 domains are altered to increase the heterodimerization of both heavy chains containing these two CH3 domains. Each of the two CH3 domains (of the two heavy chains) can be the "knob", while the other is the "hole". The introduction of a disulfide bridge further stabilizes the heterodimers (Merchant, A.M., et al., *Nature Biotech.* 16 (1998) 677-681; Atwell, S., et al., *J. Mol. Biol.* 270 (1997) 26-35) and increases the yield.

Accordingly, in a particular aspect, in the CH3 domain of the first subunit of the Fc domain of the bispecific antigen binding molecules of the invention an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an

20 amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

In a specific aspect, in the CH3 domain of the first subunit of the Fc domain ("knobs chain") the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and

30 in the CH3 domain of the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V). More particularly, in the second subunit of the Fc domain ("hole chain") additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A). More particularly, in the first subunit of the Fc domain additionally the serine residue

35 at position 354 is replaced with a cysteine residue (S354C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue

(Y349C). The introduction of these two cysteine residues results in the formation of a disulfide bridge between the two subunits of the Fc domain. The disulfide bridge further stabilizes the dimer (Carter, J Immunol Methods 248, 7-15 (2001)).

5 But also other knobs-in-holes technologies as described by EP 1870459 A1, can be used alternatively or additionally. In one embodiment the multicircular fusion polypeptide as reported herein comprises the R409D and K370E mutations in the CH3 domain of the “knobs chain” and the D399K and E357K mutations in the CH3 domain of the “hole-chain” (numbering according to Kabat EU index).

10 In a further aspect, the bispecificantigen binding molecule may comprises the Y349C and T366W mutations in one of the two CH3 domains and the S354C, T366S, L368A and Y407V mutations in the other of the two CH3 domains, or the bispecificantigen binding molecule as reported herein comprises the Y349C and T366W mutations in one of the two CH3 domains and the S354C, T366S, L368A and Y407V mutations in the other of the two CH3 domains and additionally the R409D and K370E mutations in the CH3 domain of the “knobs chain” and the
15 D399K and E357K mutations in the CH3 domain of the “hole chain” (numbering according to the Kabat EU index).

In an alternative aspect, a modification promoting association of the first and the second subunit of the Fc domain comprises a modification mediating electrostatic steering effects, e.g. as described in PCT publication WO 2009/089004. Generally, this method involves replacement
20 of one or more amino acid residues at the interface of the two Fc domain subunits by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable.

Apart from the “knob-into-hole technology” other techniques for modifying the CH3 domains of the heavy chains to enforce heterodimerization are known in the art. These
25 technologies, especially the ones described in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012/058768, WO 2013/157954 and WO 2013/096291 are contemplated herein as alternatives to the “knob-into-hole technology” in combination with a bispecific antigen binding molecule as described herein.

30 In one aspect, charged amino acids with opposite charges at specific amino acid positions in the CH3/CH3-domain-interface between both, the first and the second heavy chain are introduced to further promote the association of the desired polypeptides. Accordingly, this aspect relates to bispecific antigen binding molecules as disclosed herein, wherein in the tertiary structure of the antibody the CH3 domain of the first heavy chain and the CH3 domain of the
35 second heavy chain an interface is formed that is located between the respective antibody CH3

domains, wherein the respective amino acid sequences of the CH3 domain of the first heavy chain and the CH3 domain of the second heavy chain each comprise a set of amino acids that is located within said interface in the tertiary structure of the circular fusion polypeptide, and wherein from the set of amino acids that is located in the interface in the CH3 domain of one heavy chain a first amino acid is substituted by a positively charged amino acid and from the set of amino acids that is located in the interface in the CH3 domain of the other heavy chain a second amino acid is substituted by a negatively charged amino acid. The bispecific antigen binding molecule according to this aspect is herein also referred to as “CH3(+/-)-engineered TNF family ligand trimer-containing antigen binding molecule” (wherein the abbreviation “+/-” stands for the oppositely charged amino acids that were introduced in the respective CH3 domains). In one aspect of said CH3(+/-)-engineered bispecific antigen binding molecule as reported herein the positively charged amino acid is selected from K, R and H, and the negatively charged amino acid is selected from E or D. In another aspect, in said CH3(+/-)-engineered bispecific antigen binding molecule as reported herein the positively charged amino acid is selected from K and R, and the negatively charged amino acid is selected from E or D. In a further aspect, in said CH3(+/-)-engineered bispecific antigen binding molecule as reported herein the positively charged amino acid is K, and the negatively charged amino acid is E. In one aspect, in said CH3(+/-)-engineered bispecific antigen binding molecule as reported herein in the CH3 domain of one heavy chain the amino acid R at position 409 is substituted by D and the amino acid K at position is substituted by E, and in the CH3 domain of the other heavy chain the amino acid D at position 399 is substituted by K and the amino acid E at position 357 is substituted by K (numbering according to Kabat EU index).

In a further aspect of the invention, the IgG1 Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fc γ receptor.

Fc domain modifications reducing Fc receptor binding and/or effector function

The bispecific antibodies of the invention may comprise as a spacer domain the heavy chain domains of an immunoglobulin molecule. For example, the Fc domain of an immunoglobulin G (IgG) molecule is a dimer, each subunit of which comprises the CH2 and CH3 IgG heavy chain constant domains. The two subunits of the Fc domain are capable of stable association with each other. The Fc region confers favorable pharmacokinetic properties to the bispecific antibodies of the invention, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however, lead to undesirable targeting of the bispecific antibodies of the invention to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Accordingly, in particular embodiments the Fc region of the bispecific antibodies of the invention exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a

native IgG Fc region, in particular an IgG1 Fc region or an IgG4 Fc region. More particularly, the Fc region is an IgG1 Fc region.

In one such aspect the Fc region (or the bispecific antigen binding molecule of the invention comprising said Fc region) exhibits less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the binding affinity to an Fc receptor, as compared to a native IgG1 Fc region (or the bispecific antigen binding molecule of the invention comprising a native IgG1 Fc region), and/or less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the effector function, as compared to a native IgG1 Fc region (or the bispecific antigen binding molecule of the invention comprising a native IgG1 Fc region). In one aspect, the Fc region (or the bispecific antigen binding molecule of the invention comprising said Fc region) does not substantially bind to an Fc receptor and/or induce effector function. In a particular aspect the Fc receptor is an Fc γ receptor. In one aspect, the Fc receptor is a human Fc receptor. In one aspect, the Fc receptor is an activating Fc receptor. In a specific aspect, the Fc receptor is an activating human Fc γ receptor, more specifically human Fc γ RIIIa, Fc γ RI or Fc γ RIIa, most specifically human Fc γ RIIIa. In one aspect, the Fc receptor is an inhibitory Fc receptor. In a specific aspect, the Fc receptor is an inhibitory human Fc γ receptor, more specifically human Fc γ RIIB. In one aspect the effector function is one or more of CDC, ADCC, ADCP, and cytokine secretion. In a particular aspect, the effector function is ADCC. In one aspect, the Fc region domain exhibits substantially similar binding affinity to neonatal Fc receptor (FcRn), as compared to a native IgG1 Fc region. Substantially similar binding to FcRn is achieved when the Fc region (or the bispecific antigen binding molecule of the invention comprising said Fc region) exhibits greater than about 70%, particularly greater than about 80%, more particularly greater than about 90% of the binding affinity of a native IgG1 Fc region (or the bispecific antigen binding molecule of the invention comprising a native IgG1 Fc region) to FcRn.

In a particular aspect, the Fc region is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc region. In a particular aspect, the Fc region of the bispecific antigen binding molecule of the invention comprises one or more amino acid mutation that reduces the binding affinity of the Fc region to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc region. In one aspect, the amino acid mutation reduces the binding affinity of the Fc region to an Fc receptor. In another aspect, the amino acid mutation reduces the binding affinity of the Fc region to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In one aspect, the bispecific antigen binding molecule of the invention comprising an engineered Fc region exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to bispecific antibodies of the invention comprising a non-engineered Fc region. In a particular aspect, the Fc

receptor is an Fc γ receptor. In other aspects, the Fc receptor is a human Fc receptor. In one aspect, the Fc receptor is an inhibitory Fc receptor. In a specific aspect, the Fc receptor is an inhibitory human Fc γ receptor, more specifically human Fc γ RIIB. In some aspects the Fc receptor is an activating Fc receptor. In a specific aspect, the Fc receptor is an activating human Fc γ receptor, more specifically human Fc γ RIIIa, Fc γ RI or Fc γ RIIa, most specifically human Fc γ RIIIa. Preferably, binding to each of these receptors is reduced. In some aspects, binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one aspect, binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc region to said receptor, is achieved when the Fc region (or the bispecific antigen binding molecule of the invention comprising said Fc region) exhibits greater than about 70% of the binding affinity of a non-engineered form of the Fc region (or the bispecific antigen binding molecule of the invention comprising said non-engineered form of the Fc region) to FcRn. The Fc region, or the bispecific antigen binding molecule of the invention comprising said Fc region, may exhibit greater than about 80% and even greater than about 90% of such affinity. In certain embodiments the Fc region of the bispecific antigen binding molecule of the invention is engineered to have reduced effector function, as compared to a non-engineered Fc region. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP), reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced dendritic cell maturation, or reduced T cell priming.

In certain aspects, provided is a bispecific antibody that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the circular fusion polypeptide lacks Fc γ R binding (hence likely lacking ADCC activity), but retains FcRn binding ability.

Accordingly, in particular aspects, the Fc domain of the bispecific antibody of the invention exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG1 Fc domain. In one aspect, the Fc does not substantially bind to an Fc receptor and/or does not induce effector function. In a particular aspect the Fc receptor is an Fc γ receptor. In one aspect, the Fc receptor is a human Fc receptor. In a specific aspect, the Fc receptor is an activating human Fc γ receptor, more specifically human Fc γ RIIIa, Fc γ RI or

FcγRIIa, most specifically human FcγRIIIa. In one aspect, the Fc domain does not induce effector function. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP),
5 reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced dendritic cell maturation, or reduced T cell priming.

In certain aspects, one or more amino acid modifications may be introduced into the Fc
10 region of the bispecific antigen binding molecule provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

In a particular aspect, the invention provides a bispecificantigen binding molecule, wherein
15 the spacer domain comprises Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcγ receptor.

In one aspect, the Fc domain of the bispecificantigen binding molecule of the invention comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is
20 present in each of the two subunits of the Fc domain. In particular, the Fc domain comprises an amino acid substitution at a position of E233, L234, L235, N297, P331 and P329 (EU numbering). In particular, the Fc domain comprises amino acid substitutions at positions 234 and 235 (EU numbering) and/or 329 (EU numbering) of the IgG heavy chains. More particularly, provided is a bispecificantigen binding molecule according to the invention which comprises an
25 Fc domain with the amino acid substitutions L234A, L235A and P329G ("P329G LALA", EU numbering according to Kabat) in the IgG heavy chains. The amino acid substitutions L234A and L235A refer to the so-called LALA mutation. The "P329G LALA" combination of amino acid substitutions almost completely abolishes Fcγ receptor binding of a human IgG1 Fc domain and is described in International Patent Appl. Publ. No. WO 2012/130831 A1 which also
30 describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions. "EU numbering" refers to the numbering according to EU index of Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

Fc domains with reduced Fc receptor binding and/or effector function also include those
35 with substitution of one or more of Fc domain residues 238, 265, 269, 270, 297, 327 and 329

(U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

In another aspect, the Fc domain is an IgG4 Fc domain. IgG4 antibodies exhibit reduced
5 binding affinity to Fc receptors and reduced effector functions as compared to IgG1 antibodies. In a more specific aspect, the Fc domain is an IgG4 Fc domain comprising an amino acid substitution at position S228 (Kabat numbering), particularly the amino acid substitution S228P. In a more specific aspect, the Fc domain is an IgG4 Fc domain comprising amino acid
10 substitutions L235E and S228P and P329G (EU numbering). Such IgG4 Fc domain mutants and their Fcγ receptor binding properties are also described in WO 2012/130831.

Mutant Fc domains can be prepared by amino acid deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing.

15 Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. A suitable such binding assay is described herein. Alternatively, binding affinity of Fc domains or cell activating
20 bispecific antigen binding molecules comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as human NK cells expressing FcγIIIa receptor.

Effector function of an Fc domain, or bispecific antibodies of the invention comprising an Fc domain, can be measured by methods known in the art. A suitable assay for measuring ADCC is described herein. Other examples of in vitro assays to assess ADCC activity of a molecule of
25 interest are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACT1™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox
30 96® non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g. in a animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

In some embodiments, binding of the Fc domain to a complement component, specifically to C1q, is reduced. Accordingly, in some embodiments wherein the Fc domain is engineered to have reduced effector function, said reduced effector function includes reduced CDC. C1q binding assays may be carried out to determine whether the bispecific antibodies of the invention is able to bind C1q and hence has CDC activity. See e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J Immunol Methods 202, 163 (1996); Cragg et al., Blood 101, 1045-1052 (2003); and Cragg and Glennie, Blood 103, 2738-2743 (2004)).

In particular aspects, the bispecific antigen binding molecule comprises all positions according to EU index of Kabat)

- i) a homodimeric Fc-region of the human IgG1 subclass optionally with the mutations P329G, L234A and L235A, or
- ii) a homodimeric Fc-region of the human IgG4 subclass optionally with the mutations P329G, S228P and L235E, or
- iii) a homodimeric Fc-region of the human IgG1 subclass optionally with the mutations P329G, L234A, L235A, I253A, H310A, and H435A, or optionally with the mutations P329G, L234A, L235A, H310A, H433A, and Y436A, or
- iv) a heterodimeric Fc-region whereof
 - a) one Fc-region polypeptide comprises the mutation T366W, and the other Fc-region polypeptide comprises the mutations T366S, L368A and Y407V, or
 - b) one Fc-region polypeptide comprises the mutations T366W and Y349C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V, and S354C, or
 - c) one Fc-region polypeptide comprises the mutations T366W and S354C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V and Y349C,
- or
- v) a heterodimeric Fc-region of the human IgG1 subclass whereof both Fc-region polypeptides comprise the mutations P329G, L234A and L235A and
 - a) one Fc-region polypeptide comprises the mutation T366W, and the other Fc-region polypeptide comprises the mutations T366S, L368A and Y407V, or
 - b) one Fc-region polypeptide comprises the mutations T366W and Y349C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V, and S354C, or
 - c) one Fc-region polypeptide comprises the mutations T366W and S354C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V and Y349C,
- or
- vi) a heterodimeric Fc-region of the human IgG4 subclass whereof both Fc-region polypeptides comprise the mutations P329G, S228P and L235E and

- a) one Fc-region polypeptide comprises the mutation T366W, and the other Fc-region polypeptide comprises the mutations T366S, L368A and Y407V, or
 - b) one Fc-region polypeptide comprises the mutations T366W and Y349C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V, and S354C, or
 - 5 c) one Fc-region polypeptide comprises the mutations T366W and S354C, and the other Fc-region polypeptide comprises the mutations T366S, L368A, Y407V and Y349C,
- or
- vii) a combination of one of i), ii), and iii) with one of vi), v) and vi).

The C-terminus of the fusion polypeptides comprised in the bispecific antibodies as
 10 reported herein can be a complete C-terminus ending with the amino acid residues PGK. The C-terminus can be a shortened C-terminus in which one or two of the C-terminal amino acid residues have been removed. In one preferred embodiment the C-terminus is a shortened C-terminus ending with the amino acid residues PG.

In some aspects, provided is a bispecific antibody wherein the one antigen binding domain
 15 capable of specific binding to a second target is an antigen binding domain capable of specific binding to a tumor associated antigen (TAA). In particular, the tumor associated antigen is Fibroblast Activation Protein (FAP). In one aspect, provided is a bispecific antibody, wherein the antigen binding domain capable of specific binding to a second target is an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP).

20 **Bispecific antibodies binding to a TNF receptor and FAP**

In some aspects, the antigen binding domain capable of specific binding to FAP comprises
 (a) a heavy chain variable region ($V_{H}FAP$) comprising (i) CDR-H1 comprising the amino acid
 sequence of SEQ ID NO:1, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:2,
 and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:3, and a light chain
 25 variable region ($V_{L}FAP$) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ
 ID NO:4, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:5, and (vi) CDR-L3
 comprising the amino acid sequence of SEQ ID NO:6, or
 (b) a heavy chain variable region ($V_{H}FAP$) comprising (i) CDR-H1 comprising the amino acid
 sequence of SEQ ID NO:9, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:10,
 30 and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain
 variable region ($V_{L}FAP$) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ
 ID NO:12, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:13, and (vi) CDR-
 L3 comprising the amino acid sequence of SEQ ID NO:14.

More particularly, 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 NO:7, 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:8, 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 NO:15, 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:16.

- In one particular aspect, the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:7 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:8. In one aspect, the antigen binding domain capable of specific binding to FAP consists of a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:7 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:8.

- In another aspect, the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:15 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:16. In one aspect, the antigen binding domain capable of specific binding to FAP consists of a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:15 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:16.

In one aspect, the bispecific antibodies provided herein bind monovalent to FAP.

Bispecific antibodies binding to OX40 and FAP

- In some aspects, provided is a bispecific antibody wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to a TNF receptor, in particular a costimulatory TNF receptor. Particularly, the costimulatory TNF receptor is OX40. In one aspect, provided is a bispecific antibody, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to OX40. Particularly, the bispecific antibody of the invention comprises two antigen binding domains capable of specific binding to OX40.

In some aspects, the antigen binding domain capable of specific binding to OX40 comprises

- (a) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:35, or
- (b) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:34, or
- (c) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:23, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:36, or
- (d) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:24, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:37, or
- (e) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (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:25, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or
- (f) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (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:26, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or

(g) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (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:27, 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:33, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:39.

In particular, the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:35.

In some aspects, the antigen binding domain capable of specific binding to OX40 comprises

- (a) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:41, or
- (b) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:42 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:43, or
- (c) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:44 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:45, or
- (d) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:46 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:47, or
- (a) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:48 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:49, or
- (a) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:50 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:51, or
- (a) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:52 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:53.

In a particular aspect, the the antigen binding domain capable of specific binding to OX40 comprises (a) a heavy chain variable region (V_HOX40) 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:40, and a light chain variable region (V_LOX40) comprising an amino acid sequence
5 that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:41.

In one particular aspect, the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V_LOX40) comprising an amino acid sequence of
10 SEQ ID NO:41. In one aspect, the antigen binding domain capable of specific binding to OX40 consists of a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:41.

In one aspect, bispecific antibodies are provided that comprise two antigen binding
15 domains capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:41.

More particularly, the present invention provides a bispecific antibody, wherein the bispecific antibody comprises
20 (a) a first fusion polypeptide 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:54, and a second fusion polypeptide 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:55,
(b) a first fusion polypeptide comprising an amino acid sequence that is at least about 95%,
25 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:56, and a second fusion polypeptide 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:57,
(c) a first fusion polypeptide comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:58, and a
30 second fusion polypeptide comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:59,
(d) a first fusion polypeptide 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:60, and a second fusion polypeptide comprising an amino acid sequence that is at least about 95%, 96%,
35 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:61,

- (e) a first fusion polypeptide 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:62, and a second fusion polypeptide 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:63,
- 5 (f) a first fusion polypeptide 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:64, and a second fusion polypeptide 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:65, or
- 10 (g) a first fusion polypeptide 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:66, and a second fusion polypeptide 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:67.

In one aspect, the bispecific antibody comprises

- (a) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:54, and a
15 second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:55,
- (b) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:56, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:57,
- (c) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:58, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:59,
- 20 (d) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:60, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:61,
- (e) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:62, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:63,
- (f) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:64, and a
25 second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:65, or
- (g) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:66, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:67.

Furthermore, the present invention provides a bispecific antibody, wherein the bispecific antibody comprises

- 30 (a) a first fusion polypeptide 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:116, and a second fusion polypeptide 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:117,
- (b) a first fusion polypeptide comprising an amino acid sequence that is at least about 95%,
35 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:118, and a

second fusion polypeptide 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:119,

(c) a first fusion polypeptide 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:120, and a

5 second fusion polypeptide 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:121,

(d) a first fusion polypeptide 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:122, and a second fusion polypeptide comprising an amino acid sequence that is at least about 95%, 96%,

10 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:123,

(e) a first fusion polypeptide 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:124, and a second fusion polypeptide 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:125,

15 (f) a first fusion polypeptide 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:126, a second fusion polypeptide 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:127, and a light chain that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid

20 sequence of SEQ ID NO:128,

(g) a first fusion polypeptide 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:129, and a second fusion polypeptide 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:130,

25 (h) a first fusion polypeptide 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:131, and a second fusion polypeptide 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:132, or

(i) a first fusion polypeptide comprising an amino acid sequence that is at least about 95%, 30 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:133, and a second fusion polypeptide 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:134.

In one aspect, the bispecific antibody comprises

(a) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:116, and a 35 second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:117,

(b) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:118, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:119,

- (c) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:120, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:121,
- (d) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:122, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:123,
- 5 (e) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:124, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:125,
- (f) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:126, a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:127, and a light chain comprising the amino acid sequence of SEQ ID NO:128,
- 10 (g) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:129, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:130,
- (h) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:131, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:132, or
- (i) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:133, and a
- 15 second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:134.

Bispecific antibodies binding to 4-1BB and FAP

In some aspects, provided is a bispecific antibody wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to a TNF receptor, wherein the costimulatory TNF receptor is 4-1BB. In one aspect,

20 provided is a bispecific antibody, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to 4-1BB. Particularly, the bispecific antibody of the invention comprises two antigen binding domains capable of specific binding to 4-1BB.

In some aspects, the antigen binding domain capable of specific binding to 4-1BB a heavy chain variable region (V_H4-1BB) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:137, and a light chain variable region (V_L4-1BB) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and (vi) CDR-L3

30 comprising the amino acid sequence of SEQ ID NO:140. In one aspect, the antigen binding domain capable of specific binding to 4-1BB comprises a heavy chain variable region (V_H4-1BB) 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:141, and a light chain variable region (V_L4-1BB) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or

35 100% identical to the amino acid sequence of SEQ ID NO:142.

In one particular aspect, the antigen binding domain capable of specific binding to 4-1BB comprises a heavy chain variable region (V_H4-1BB) comprising an amino acid sequence of SEQ ID NO:141 and a light chain variable region (V_L4-1BB) comprising an amino acid sequence of SEQ ID NO:142. In one aspect, the antigen binding domain capable of specific binding to 4-1BB
5 consists of a heavy chain variable region (V_H4-1BB) comprising an amino acid sequence of SEQ ID NO:141 and a light chain variable region (V_L4-1BB) comprising an amino acid sequence of SEQ ID NO:142.

Particularly, the present invention provides a bispecific antibody, wherein the bispecific antibody comprises
10 (a) a first fusion polypeptide 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:143, and a second fusion polypeptide 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:144, or
(b) a first fusion polypeptide comprising an amino acid sequence that is at least about 95%,
15 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:145, and a second fusion polypeptide 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:146.

In one aspect, the bispecific antibody comprises
(a) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:143, and a
20 second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:144, or
(b) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:145, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:146.

Bispecific antibodies binding to CD40 and FAP

In some aspects, provided is a bispecific antibody wherein the antigen binding domain
25 capable of specific binding to a first target is an antigen binding domain capable of specific binding to a TNF receptor, wherein the costimulatory TNF receptor is CD40. In one aspect, provided is a bispecific antibody, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to CD40. Particularly, the bispecific antibody of the invention comprises two antigen binding domains
30 capable of specific binding to CD40.

In some aspects, the antigen binding domain capable of specific binding to CD40 comprises a heavy chain variable region (VHCD40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:147, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:148, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:149, and
35 a light chain variable region (VLCD40) comprising (iv) CDR-L1 comprising the amino acid

sequence of SEQ ID NO:150, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:151, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:152. In one aspect, the antigen binding domain capable of specific binding to CD40 comprises a heavy chain variable region (VHCD40) 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:153, and a light chain variable region (VLCD40) 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:154.

- In another aspect, the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to CD40 comprises
- 10 (i) a heavy chain variable region (VHCD40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169 and SEQ ID NO:170, and a light chain variable region (VLCD40) comprising the 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, or
 - 15 (ii) a heavy chain variable region (VHCD40) 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:179 and SEQ ID NO:180, and a light chain variable region (VLCD40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, and SEQ ID NO:184.

20 In one aspect, the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to CD40 comprises

- (a) a heavy chain variable region (VHCD40) comprising an amino acid sequence of SEQ ID NO:153, and a light chain variable region (VLCD40) comprising an amino acid sequence of SEQ ID NO:154, or
- 25 (b) a heavy chain variable region (VHCD40) comprising an amino acid sequence of SEQ ID NO:167, and a light chain variable region (VLCD40) comprising an amino acid sequence of SEQ ID NO:171.

Particularly, the present invention provides a bispecific antibody, wherein the bispecific antibody comprises

- 30 (a) a first fusion polypeptide 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:155, and a second fusion polypeptide 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:156,
- (b) a first fusion polypeptide 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:157, and a
- 35

second fusion polypeptide 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:158,

(c) a first fusion polypeptide 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:159, and a

5 second fusion polypeptide 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:160,

(d) a first fusion polypeptide 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:161, and a second fusion polypeptide comprising an amino acid sequence that is at least about 95%, 96%,

10 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:162,

(e) a first fusion polypeptide 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:163, and a second fusion polypeptide 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:164, or

15 (f) a first fusion polypeptide 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:165, and a second fusion polypeptide 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:166.

In one aspect, the the bispecific antibody comprises

20 (a) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:155, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:156,

(b) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:157, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:158,

(c) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:159, and a
25 second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:160,

(d) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:161, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:162,

(e) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:163, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:164, or

30 (f) a first fusion polypeptide comprising an amino acid sequence of SEQ ID NO:165, and a second fusion polypeptide comprising an amino acid sequence of SEQ ID NO:166.

Modifications in the Fab domains

In one aspect, the invention relates to a bispecific antibody comprising (a) two Fab fragments capable of specific binding to OX40 and (b) one Fab fragment capable of specific

35 binding to FAP, wherein in one of (a) and (b) in the Fab fragments either the variable domains

VH and VL or the constant domains CH1 and CL are exchanged. The bispecific antibodies are prepared according to the Crossmab technology.

Multispecific antibodies with a domain replacement/exchange in one binding arm (CrossMabVH-VL or CrossMabCH-CL) are described in detail in WO2009/080252 and
5 Schaefer, W. et al, PNAS, 108 (2011) 11187-1191. They clearly reduce the byproducts caused by the mismatch of a light chain against a first antigen with the wrong heavy chain against the second antigen (compared to approaches without such domain exchange).

In one aspect, the invention relates to a bispecific antibody comprising (a) two Fab fragments capable of specific binding to OX40 and (b) a cross-Fab fragment capable of specific
10 binding to FAP, wherein the constant domains CL (Ckappa) and CH1 are replaced by each other so that the CH1 domain is fused to the VL domain and the CL domain is fused to the VH domain.

In another aspect, the invention relates to a bispecific antibody, comprising (a) two cross-Fab fragments capable of specific binding to OX40, wherein the VH domain is fused to the CL (Ckappa) domain and the VL domain is fused to the CH1 domain and (b) a Fab fragment capable
15 of specific binding to FAP.

In another aspect, and to further improve correct pairing, the bispecific antibody can contain different charged amino acid substitutions (so-called "charged residues"). These modifications are introduced in the crossed or non-crossed CH1 and CL domains. In a particular aspect, the invention relates to a bispecific antibody, wherein in one of CL domains the amino
20 acid at position 123 (EU numbering) has been replaced by arginine (R) and the amino acid at position 124 (EU numbering) has been substituted by lysine (K) (positive charges) and wherein in one of the CH1 domains the amino acids at position 147 (EU numbering) and at position 213 (EU numbering) have been substituted by glutamic acid (E) (negative charges).

More particularly, the invention relates to a bispecific antigen binding molecule
25 comprising a Fab, wherein in the CL domain the amino acid at position 123 (EU numbering) has been replaced by arginine (R) and the amino acid at position 124 (EU numbering) has been substituted by lysine (K), and wherein in the CH1 domain the amino acids at position 147 (EU numbering) and at position 213 (EU numbering) have been substituted by glutamic acid (E).

Accordingly, in some embodiments one or more of the Fab fragments (e.g. Fab fragments
30 capable of specific binding to OX40) of the bispecific antigen binding molecule of the present invention comprise a CL domain comprising an arginine (R) at amino acid at position 123 (EU numbering) and a lysine (K) at amino acid at position 124 (EU numbering), and a CH1 domain comprising a glutamic acid (E) at amino acid at position 147 (EU numbering) and a glutamic acid (E) at amino acid at position 213 (EU numbering).

Polynucleotides

The invention further provides isolated nucleic acid encoding a bispecific antibody of the invention as described herein, or a fragment thereof.

The isolated nucleic acid encoding a bispecific antibody of the invention may be expressed
5 as a single polynucleotide that encodes the entire antigen binding molecule or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional antigen binding molecule. When co-expressed, the fusion polypeptides will associate to form the antigen binding domain capable of specific binding to the second target (e.g. FAP).
10 The antigen binding domains capable of specific binding to the first target (e.g. OX40) may be encoded by one polynucleotide. When co-expressed, the fusion polypeptides will associate to form the bispecific antibody.

In certain embodiments the polynucleotide or nucleic acid is DNA. In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA
15 (mRNA). RNA of the present invention may be single stranded or double stranded.

According to another aspect of the invention, there is provided an isolated polynucleotide encoding a fusion polypeptide as described herein before. The invention further provides a vector, particularly an expression vector, comprising the isolated polynucleotide of the invention and a host cell comprising the isolated polynucleotide or the vector of the invention. In some
20 embodiments the host cell is a eukaryotic cell, particularly a mammalian cell.

In another aspect, provided is a method for producing the bispecific antibody of the invention, comprising the steps of (i) culturing the host cell of the invention under conditions suitable for expression of said antibody, and (ii) isolating said bispecific antibody. The invention also encompasses a bispecific antibody produced by the method of the invention.

25 Recombinant Methods

Bispecific antigen binding molecules of the invention may be obtained, for example, by solid-state peptide synthesis (e.g. Merrifield solid phase synthesis) or recombinant production. For recombinant production one or more polynucleotide encoding the antigen binding molecule or polypeptide fragments thereof, e.g., as described above, is isolated and inserted into one or
30 more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In one aspect of the invention, a vector, preferably an expression vector, comprising one or more of the polynucleotides of the invention is provided. Methods which are well known to those skilled in the art can be used to

construct expression vectors containing the coding sequence of the bispecific antigen binding molecule (fragment) along with appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, N.Y. (1989); and Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, N.Y. (1989). The expression vector can be part of a plasmid, virus, or may be a nucleic acid fragment. The expression vector includes an expression cassette into which the polynucleotide encoding the bispecific antigen binding molecule or polypeptide fragments thereof (i.e. the coding region) is cloned in operable association with a promoter and/or other transcription or translation control elements. As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5' and 3' untranslated regions, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g. on a single vector, or in separate polynucleotide constructs, e.g. on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a polynucleotide encoding the bispecific antigen binding molecule of the invention or polypeptide fragments thereof, or variants or derivatives thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain. An operable association is when a coding region for a gene product, e.g. a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for

example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription.

Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without
5 limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (e.g. the immediate early promoter, in conjunction with intron-A), simian virus 40 (e.g. the early promoter), and retroviruses (such as, e.g. Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and
10 rabbit α -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as inducible promoters (e.g. promoters inducible tetracyclins). Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination
15 codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence). The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

Polynucleotide and nucleic acid coding regions of the present invention may be associated
20 with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the bispecific antigen binding molecule or polypeptide fragments thereof is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding a
25 bispecific antigen binding molecule of the invention or polypeptide fragments thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a
30 signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the translated polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, e.g. an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian
35 signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β -glucuronidase.

DNA encoding a short protein sequence that could be used to facilitate later purification (e.g. a histidine tag) or assist in labeling the fusion protein may be included within or at the ends of the polynucleotide encoding a bispecific antigen binding molecule of the invention or polypeptide fragments thereof.

5 In a further aspect of the invention, a host cell comprising one or more polynucleotides of the invention is provided. In certain embodiments a host cell comprising one or more vectors of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in combination, described herein in relation to polynucleotides and vectors, respectively. In one aspect, a host cell comprises (e.g. has been transformed or transfected with)
10 a vector comprising a polynucleotide that encodes (part of) a bispecific antigen binding molecule of the invention. As used herein, the term "host cell" refers to any kind of cellular system which can be engineered to generate the fusion proteins of the invention or fragments thereof. Host cells suitable for replicating and for supporting expression of antigen binding molecules are well known in the art. Such cells may be transfected or transduced as appropriate with the particular
15 expression vector and large quantities of vector containing cells can be grown for seeding large scale fermenters to obtain sufficient quantities of the antigen binding molecule for clinical applications. Suitable host cells include prokaryotic microorganisms, such as *E. coli*, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO), human embryonic kidney (HEK) cells, insect cells, or the like. For example, polypeptides may be produced in bacteria in
20 particular when glycosylation is not needed. After expression, the polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized", resulting in the production of a polypeptide
25 with a partially or fully human glycosylation pattern. See Gerngross, *Nat Biotech* 22, 1409-1414 (2004), and Li et al., *Nat Biotech* 24, 210-215 (2006).

Suitable host cells for the expression of (glycosylated) polypeptides are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in
30 conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts. See e.g. US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of
35 useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham et al., *J Gen Virol* 36, 59 (1977)), baby hamster kidney cells (BHK), mouse sertoli cells (TM4 cells as described,

e.g., in Mather, Biol Reprod 23, 243-251 (1980)), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMT 060562), TRI cells (as described, e.g., in Mather et al., 5 Annals N.Y. Acad Sci 383, 44-68 (1982)), MRC 5 cells, and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including dhfr- CHO cells (Urlaub et al., Proc Natl Acad Sci USA 77, 4216 (1980)); and myeloma cell lines such as YO, NS0, P3X63 and Sp2/0. For a review of certain mammalian host cell lines suitable for protein production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, 10 Totowa, NJ), pp. 255-268 (2003). Host cells include cultured cells, e.g., mammalian cultured cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one embodiment, the host cell is a eukaryotic cell, preferably a mammalian cell, such as a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g., Y0, 15 NS0, Sp20 cell). Standard technologies are known in the art to express foreign genes in these systems. Cells expressing a polypeptide comprising either the heavy or the light chain of an antigen binding domain, may be engineered so as to also express the other of the immunoglobulin chains such that the expressed product is an antigen binding domain that has both a heavy and a light chain.

20 In another aspect, provided is a method for producing the bispecific antibody of the invention, comprising the steps of (i) culturing the host cell of the invention under conditions suitable for expression of said bispecific antibody, and (ii) isolating said bispecific antibody from the host cell or host cell culture medium.

The components of the bispecific antibody are genetically fused to each other. Bispecific 25 antigen binding molecules can be designed such that its components are fused directly to each other or indirectly through a linker sequence. The composition and length of the linker may be determined in accordance with methods well known in the art and may be tested for efficacy. Examples of linker sequences between different components of bispecific antigen binding molecules are found in the sequences provided herein. Additional sequences may also be 30 included to incorporate a cleavage site to separate the individual components of the fusion if desired, for example an endopeptidase recognition sequence.

In certain aspects, the antigen binding domain capable of specific binding to FAP (e.g. Fab fragments or scFv) forming part of the antibody comprises at least an immunoglobulin variable region capable of binding to FAP. Similarly, in certain aspects, the moieties capable of specific 35 binding to OX40 (e.g. Fab fragments or scFv) forming part of the bispecific antibody comprise at least an immunoglobulin variable region capable of binding to OX40. Variable regions can form

part of and be derived from naturally or non-naturally occurring antibodies and fragments thereof. Methods to produce polyclonal antibodies and monoclonal antibodies are well known in the art (see e.g. Harlow and Lane, "Antibodies, a laboratory manual", Cold Spring Harbor Laboratory, 1988). Non-naturally occurring antibodies can be constructed using solid phase-peptide synthesis, 5 can be produced recombinantly (e.g. as described in U.S. patent No. 4,186,567) or can be obtained, for example, by screening combinatorial libraries comprising variable heavy chains and variable light chains (see e.g. U.S. Patent. No. 5,969,108 to McCafferty).

In certain aspects, the antigen binding domains capable of specific binding to the relevant target (e.g. Fab fragments or scFv) comprised in the antigen binding molecules of the present 10 invention are engineered to have enhanced binding affinity according to, for example, the methods disclosed in PCT publication WO 2012/020006 (see Examples relating to affinity maturation) or U.S. Pat. Appl. Publ. No. 2004/0132066. The ability of the antigen binding molecules of the invention to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of 15 skill in the art, e.g. surface plasmon resonance technique (Liljeblad, et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)). Competition assays may be used to identify an antigen binding molecule that competes with a reference antibody for binding to a particular antigen. In certain embodiments, such a competing antigen binding molecule binds to the same epitope (e.g. a linear or a conformational epitope) that is 20 bound by the reference antigen binding molecule. Detailed exemplary methods for mapping an epitope to which an antigen binding molecule binds are provided in Morris (1996) "Epitope Mapping Protocols", in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ). In an exemplary competition assay, immobilized antigen is incubated in a solution comprising a first labeled antigen binding molecule that binds to the antigen and a second unlabeled antigen 25 binding molecule that is being tested for its ability to compete with the first antigen binding molecule for binding to the antigen. The second antigen binding molecule may be present in a hybridoma supernatant. As a control, immobilized antigen is incubated in a solution comprising the first labeled antigen binding molecule but not the second unlabeled antigen binding molecule. After incubation under conditions permissive for binding of the first antibody to the antigen, 30 excess unbound antibody is removed, and the amount of label associated with immobilized antigen is measured. If the amount of label associated with immobilized antigen is substantially reduced in the test sample relative to the control sample, then that indicates that the second antigen binding molecule is competing with the first antigen binding molecule for binding to the antigen. See Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring 35 Harbor Laboratory, Cold Spring Harbor, NY).

Bispecific antibodies of the invention prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion exchange

chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity etc., and will be apparent to those having skill in the art. For affinity chromatography purification an antibody, ligand, receptor or antigen can be used to which the bispecific antigen binding molecule binds. For example, for affinity chromatography purification of fusion proteins of the invention, a matrix with protein A or protein G may be used. Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate an antigen binding molecule essentially as described in the Examples. The purity of the bispecific antigen binding molecule or fragments thereof can be determined by any of a variety of well-known analytical methods including gel electrophoresis, high pressure liquid chromatography, and the like. For example, the bispecific antigen binding molecules expressed as described in the Examples were shown to be intact and properly assembled as demonstrated by reducing and non-reducing SDS-PAGE.

The invention also encompasses a bispecific antibodies produced by the methods of the invention.

Assays

The bispecific antigen binding molecules provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Affinity assays

The affinity of the bispecific antigen binding molecule provided herein for OX40 or FAP can be determined in accordance with the methods set forth in the Examples by surface plasmon resonance (SPR), using standard instrumentation such as a BIAcore instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. A specific illustrative and exemplary embodiment for measuring binding affinity is described in **Example 3**. According to one aspect, K_D is measured by surface plasmon resonance using a BIACORE® T200 machine (GE Healthcare) at 25 °C.

2. Binding assays and other assays

Binding of the bispecific antigen binding molecule provided herein to the corresponding OX40 and/or FAP expressing cells may be evaluated using cell lines expressing the particular receptor or target antigen, for example by flow cytometry (FACS). In one aspect, fresh peripheral blood mononuclear cells (PBMCs) expressing OX40 are used in the binding assay. These cells are used directly after isolation (naïve PMBCs) or after stimulation (activated

PMBCs). A specific illustrative and exemplary embodiment for measuring binding to OX40 is described in **Example 4.1**.

In a further aspect, cancer cell lines expressing FAP were used to demonstrate the binding of the bispecific antibodies to FAP (see **Example 4.2**).

5 In another aspect, competition assays may be used to identify an antigen binding molecule that competes with a specific antibody or antigen binding molecule for binding to FAP or OX40, respectively. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by a specific anti-FAP antibody or a specific anti-OX40 antibody. Detailed exemplary methods for mapping an epitope to which an antibody
10 binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

3. Activity assays

In one aspect, assays are provided for identifying bispecific antigen binding molecules that bind to FAP and to OX40 having biological activity. Biological activity may include, e.g.,
15 agonistic signalling through OX40 on cells expressing OX40. Bispecific antigen binding molecules identified by the assays as having such biological activity *in vitro* are also provided. In particular, a reporter cell assay detecting NFκB activation in Hela cells expressing human OX40 and co-cultured with FAP-expressing tumor cells is provided (see e.g. **Example 5.1**).

In another aspect, assays are provided for identifying bispecific antigen binding molecules
20 that bind to FAP and to 4-1BB having biological activity. In particular, a reporter cell assay detecting NF-κB activation in human 4-1BB and NFκB-luciferase reporter gene expressing reporter cell line Jurkat-hu4-1BB-NFκB-luc2 is provided (see e.g. **Example 7.2**).

In another aspect, assays are provided for identifying bispecific antigen binding molecules that bind to FAP and to CD40 having biological activity. In particular, a method of measuring
25 the activation of human B cells by FAP-targeted anti-human CD40 binding molecules using FAP-coated Dynabeads® as source of antigen is provided (see e.g. **Example 10.1**).

In certain aspects, bispecific antigen binding molecules of the invention are tested for such biological activity. Assays for detecting the biological activity of the molecules of the invention are those described in **Example 5**. Furthermore, assays for detecting cell lysis (e.g. by
30 measurement of LDH release), induced apoptosis kinetics (e.g. by measurement of Caspase 3/7 activity) or apoptosis (e.g. using the TUNEL assay) are well known in the art. In addition, the biological activity of such complexes can be assessed by evaluating their effects on survival, proliferation and lymphokine secretion of various lymphocyte subsets such as NK cells, NKT-

cells or $\gamma\delta$ T-cells or assessing their capacity to modulate phenotype and function of antigen presenting cells such as dendritic cells, monocytes/macrophages or B-cells.

Pharmaceutical Compositions, Formulations and Routes of Administration

In a further aspect, the invention provides pharmaceutical compositions comprising any of the bispecific antibodies provided herein, e.g., for use in any of the below therapeutic methods.
5 In one embodiment, a pharmaceutical composition comprises any of the bispecific antibodies provided herein and at least one pharmaceutically acceptable excipient. In another embodiment, a pharmaceutical composition comprises any of the bispecific antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

10 Pharmaceutical compositions of the present invention comprise a therapeutically effective amount of one or more bispecific antibodies dissolved or dispersed in a pharmaceutically acceptable excipient. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that are generally non-toxic to recipients at the dosages and concentrations employed, i.e. do not produce an adverse, allergic or other untoward reaction
15 when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one bispecific antibody and optionally an additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. In particular, the compositions are lyophilized
20 formulations or aqueous solutions. As used herein, "pharmaceutically acceptable excipient" includes any and all solvents, buffers, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g. antibacterial agents, antifungal agents), isotonic agents, salts, stabilizers and combinations thereof, as would be known to one of ordinary skill in the art.

Parenteral compositions include those designed for administration by injection, e.g.
25 subcutaneous, intradermal, intralesional, intravenous, intraarterial intramuscular, intrathecal or intraperitoneal injection. For injection, the bispecific antibodies of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the fusion proteins
30 may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. Sterile injectable solutions are prepared by incorporating the fusion proteins of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated below, as required. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. Generally, dispersions are prepared by incorporating the various
35 sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium

and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium
5 should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein. Suitable pharmaceutically
10 acceptable excipients include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight
15 (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium;
20 metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Aqueous injection suspensions may contain compounds which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, dextran, or the like. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.
25 Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl esters or triglycerides, or liposomes.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose
30 or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (18th Ed. Mack Printing Company, 1990). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include
35 semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, e.g. films, or microcapsules. In particular embodiments, prolonged absorption of an injectable composition can be brought about by the

use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

Exemplary pharmaceutically acceptable excipients herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

In addition to the compositions described previously, the fusion proteins may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the fusion proteins may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Pharmaceutical compositions comprising the fusion proteins of the invention may be manufactured by means of conventional mixing, dissolving, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

The bispecific antibodies may be formulated into a composition in a free acid or base, neutral or salt form. Pharmaceutically acceptable salts are salts that substantially retain the biological activity of the free acid or base. These include the acid addition salts, e.g. those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms.

The composition herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

- 5 The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

Therapeutic methods and compositions

- Any of the bispecific antibodies provided herein may be used in therapeutic methods. For use in therapeutic methods, the antigen binding molecules of the invention can be formulated,
10 dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

- 15 In one aspect, the bispecific antibodies of the invention are provided for use as a medicament. In further aspects, the bispecific antigen binding molecules of the invention are provided for use in treating a disease, in particular for use in the treatment of cancer. In certain embodiments, the bispecific antibodies of the invention are provided for use in a method of treatment. In one embodiment, the invention provides a bispecific antibody as described herein
20 for use in the treatment of a disease in an individual in need thereof. In certain embodiments, the invention provides a bispecific antibody for use in a method of treating an individual having a disease comprising administering to the individual a therapeutically effective amount of the bispecific antigen binding molecule. In certain embodiments the disease to be treated is cancer. In certain embodiments the disease to be treated is a proliferative disorder, particularly cancer.
25 Examples of cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone cancer, and kidney cancer. Other cell proliferation disorders that can be treated using a bispecific antigen binding molecule
30 of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases. In certain
35 embodiments the cancer is chosen from the group consisting of renal cell cancer, skin cancer,

lung cancer, colorectal cancer, breast cancer, brain cancer, head and neck cancer. The subject, patient, or "individual" in need of treatment is typically a mammal, more specifically a human.

Also encompassed by the invention is the bispecific antibody of the invention, or the pharmaceutical composition of the invention, for use in up-regulating or prolonging cytotoxic T
5 cell activity.

In a further aspect, the invention provides for the use of a bispecific antibody of the invention in the manufacture or preparation of a medicament for the treatment of a disease in an individual in need thereof. In one aspect, the medicament is for use in a method of treating a disease comprising administering to an individual having the disease a therapeutically effective
10 amount of the medicament. In certain embodiments the disease to be treated is a proliferative disorder, particularly cancer. Examples of cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone
15 cancer, and kidney cancer. Other cell proliferation disorders that can be treated using a bispecific antigen binding molecule of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen,
20 thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases. In certain embodiments the cancer is chosen from the group consisting of renal cell cancer, skin cancer, lung cancer, colorectal cancer, breast cancer, brain cancer, head and neck cancer. A skilled artisan readily recognizes that in many cases the bispecific antigen binding molecule may not provide a cure but may only provide partial benefit. In some
25 embodiments, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some embodiments, an amount of bispecific antibody that provides a physiological change is considered an "effective amount" or a "therapeutically effective amount". In any of the above embodiments the individual is preferably a mammal, particularly a human.

In a further aspect, the invention relates to the use of a bispecific antibody as described
30 herein in the manufacture or preparation of a medicament for the treatment of infectious diseases, in particular for the treatment of viral infections or for the treatment of autoimmune diseases, for example Lupus disease.

In a further aspect, the invention provides a method for treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a bispecific
35 antigen binding molecule of the invention. In one embodiment a composition is administered to

said individual, comprising a fusion protein of the invention in a pharmaceutically acceptable form. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional
5 therapeutic agent, e.g. an anti-cancer agent if the disease to be treated is cancer. An “individual” according to any of the above embodiments may be a mammal, preferably a human.

For the prevention or treatment of disease, the appropriate dosage of a bispecific antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the route of administration,
10 the body weight of the patient, the type of fusion protein, the severity and course of the disease, whether the fusion protein is administered for preventive or therapeutic purposes, previous or concurrent therapeutic interventions, the patient's clinical history and response to the fusion protein, and the discretion of the attending physician. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a
15 composition and appropriate dose(s) for the individual subject. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

The bispecific antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g.
20 0.1 mg/kg – 10 mg/kg) of the antigen binding molecule can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a
25 desired suppression of disease symptoms occurs. One exemplary dosage of the fusion protein would be in the range from about 0.005 mg/kg to about 10 mg/kg. In other examples, a dose may also comprise from about 1 µg/kg body weight, about 5 µg/kg body weight, about 10 µg/kg body weight, about 50 µg/kg body weight, about 100 µg/kg body weight, about 200 µg/kg body weight, about 350 µg/kg body weight, about 500 µg/kg body weight, about 1 mg/kg body weight,
30 about 5 mg/kg body weight, about 10 mg/kg body weight, about 50 mg/kg body weight, about 100 mg/kg body weight, about 200 mg/kg body weight, about 350 mg/kg body weight, about 500 mg/kg body weight, to about 1000 mg/kg body weight or more per administration, and any range derivable therein. In examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg body weight to about 100 mg/kg body weight, about 5 µg/kg body weight to
35 about 500 mg/kg body weight etc., can be administered, based on the numbers described above. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 5.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered

intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the bispecific antigen binding molecule). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored
5 by conventional techniques and assays.

The bispecific antibody of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the bispecific antigen binding molecules of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. Determination of a therapeutically
10 effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture.
15 Such information can be used to more accurately determine useful doses in humans. Initial dosages can also be estimated from *in vivo* data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data. Dosage amount and interval may be adjusted individually to provide plasma levels of the bispecific antigen binding molecules which are sufficient to
20 maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.5 to 1 mg/kg/day. Therapeutically effective plasma levels may be achieved by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC.

In cases of local administration or selective uptake, the effective local concentration of the
25 bispecific antigen binding molecules may not be related to plasma concentration. One skilled in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

A therapeutically effective dose of the bispecific antibody described herein will generally provide therapeutic benefit without causing substantial toxicity. Toxicity and therapeutic efficacy
30 of a fusion protein can be determined by standard pharmaceutical procedures in cell culture or experimental animals. Cell culture assays and animal studies can be used to determine the LD_{50} (the dose lethal to 50% of a population) and the ED_{50} (the dose therapeutically effective in 50% of a population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which can be expressed as the ratio LD_{50}/ED_{50} . Bispecific antigen binding molecules that exhibit
35 large therapeutic indices are preferred. In one embodiment, the bispecific antigen binding

molecule according to the present invention exhibits a high therapeutic index. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages suitable for use in humans. The dosage lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this
5 range depending upon a variety of factors, e.g., the dosage form employed, the route of administration utilized, the condition of the subject, and the like. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al., 1975, in: The Pharmacological Basis of Therapeutics, Ch. 1, p. 1, incorporated herein by reference in its entirety).

10 The attending physician for patients treated with the bispecific antigen binding molecules of the invention will know how and when to terminate, interrupt, or adjust administration due to toxicity, organ dysfunction, and the like. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary
15 with the severity of the condition to be treated, with the route of administration, and the like. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient.

Other agents and treatments

20 The bispecific antibodies of the invention may be administered in combination with one or more other agents in therapy. For instance, a bispecific antibody of the invention may be co-administered with at least one additional therapeutic agent. The term "therapeutic agent" encompasses any agent that can be administered for treating a symptom or disease in an individual in need of such treatment. Such additional therapeutic agent may comprise any active
25 ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. In certain embodiments, an additional therapeutic agent is another anti-cancer agent.

Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of fusion
30 protein used, the type of disorder or treatment, and other factors discussed above. The bispecific antigen binding molecules are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

Such combination therapies noted above encompass combined administration (where two
35 or more therapeutic agents are included in the same or separate compositions), and separate

administration, in which case, administration of the bispecific antigen binding molecule of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

Articles of Manufacture

5 In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The
10 container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper that is pierceable by a hypodermic injection needle). At least one active agent in the composition is a bispecific antibody as described herein.

15 The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a bispecific antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of
20 manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition.

Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may
25 further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Table B. Sequences

SEQ ID NO:	Name	Sequence
1	FAP(4B9) CDR-H1	SYAMS
2	FAP(4B9) CDR-H2	AIIGSGASTYYADSVKG
3	FAP(4B9) CDR-H3	GWFGGFNY
4	FAP(4B9) CDR-L1	RASQSVTSSYLA
5	FAP(4B9) CDR-L2	VGSRRAT

SEQ ID NO:	Name	Sequence
6	FAP(4B9) CDR-L3	QQGIMLPPT
7	FAP(4B9) VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSW VRQAPGKGLEWVSAIIGSGASTYYADSVKGRFTISR NSKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYW GQGTLVTVSS
8	FAP(4B9) VL	EIVLTQSPGTLISLSPGERATLSCRASQSVTSSYLAWY QQKPGQAPRLINVGSRRTATGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIK
9	FAP (28H1) CDR-H1	SHAMS
10	FAP (28H1) CDR-H2	AIWASGEQYYADSVKG
11	FAP (28H1) CDR-H3	GWLGNFY
12	FAP (28H1) CDR-L1	RASQSVRSYLA
13	FAP (28H1) CDR-L2	GASTRAT
14	FAP (28H1) CDR-L3	QQGQVIPPT
15	FAP(28H1) VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSW VRQAPGKGLEWVSAIWASGEQYYADSVKGRFTISR NSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFYD GQGTLVTVSS
16	FAP(28H1) VL	EIVLTQSPGTLISLSPGERATLSCRASQSVRSYLAWY QQKPGQAPRLIIGASTRATGIPDRFSGSGSGTDFTL SRLEPEDFAVYYCQQGQVIPPTFGQGTKVEIK
17	OX40(8H9,49B4,1G4, 20B7) CDR-H1	SYAIS
18	OX40(CLC-563, CLC- 564, 17A9) CDR-H1	SYAMS
19	OX40(8H9,49B4,1G4, 20B7) CDR-H2	GIPIFGTANYAQKFQG
20	OX40(CLC-563, CLC- 564, 17A9) CDR-H2	AISGSGGSTYYADSVKG
21	OX40(8H9) CDR-H3	EYGWMDY
22	OX40(49B4) CDR-H3	EYYRGPYDY
23	OX40(1G4) CDR-H3	EYGSMYD
24	OX40(20B7) CDR-H3	VNYPYSYWGDFDY
25	OX40(CLC-563) CDR-H3	DVGAFDY
26	OX40(CLC-564) CDR-H3	DVGPFYD
27	OX40(17A9)-CDR-H3	VFYRGGVSMYD
28	OX40(8H9,49B4,1G4, 20B7) CDR-L1	RASQSISSWLA
29	OX40(CLC-563, CLC564) CDR-L1	RASQSVSSSYLA
30	OX40(17A9) CDR-L1	QGDSLRSYYAS
31	OX40(8H9,49B4,1G4, 20B7) CDR-L2	DASSLES
32	OX40(CLC-563, CLC564) CDR-L2	GASSRAT
33	OX40(17A9) CDR-L2	GKNNRPS
34	OX40(8H9) CDR-L3	QQYLTYSRFT

SEQ ID NO:	Name	Sequence
35	OX40(49B4) CDR-L3	QQYSSQPYT
36	OX40(1G4) CDR-L3	QQYISYSMLT
37	OX40(20B7) CDR-L3	QQYQAFSLT
38	OX40(CLC-563, CLC-564) CDR-L3	QQYGSSPLT
39	OX40(17A9) CDR-L3	NSRVMPHNRV
40	OX40(49B4) VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDTAVYYCAREYYRGPYDY WGQGTTVTSS
41	OX40(49B4) VL	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQ QKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLT SSLQPDDEFATYYCQQYSSQPYTFGQGTKVEIK
42	OX40(8H9) VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDTAVYYCAREYGMWDYWG QGTTVTSS
43	OX40(8H9) VL	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQ QKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLT SSLQPDDEFATYYCQQYLYSRFTFGQGTKVEIK
44	OX40(1G4) VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDTAVYYCAREYGSMDYWG QGTTVTSS
45	OX40(1G4) VL	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQ QKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLT SSLQPDDEFATYYCQQYISYSMLTFGQGTKVEIK
46	OX40(20B7) VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDTAVYYCARVNYPYSYWG DEDYWGQGTTVTSS
47	OX40(20B7) VL	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQ QKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLT SSLQPDDEFATYYCQQYQAFSLTFGQGTKVEIK
48	OX40(CLC-563) VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSW VRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCALDVGAFDYWG QGALVTSS
49	OX40(CLC-563) VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWY QQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLT ISRLEPEDFAVYYCQQYGSSPLTFGQGTKVEIK
50	OX40(CLC-564) VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSW VRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCAFDVGPFDYWG QGTILVTSS
51	OX40(CLC-564) VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWY QQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLT ISRLEPEDFAVYYCQQYGSSPLTFGQGTKVEIK
52	OX40(17A9) VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSW VRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISR

SEQ ID NO:	Name	Sequence
		DNSKNTLYLQMNSLRAEDTAVYYCARV <u>FYRGGVSM</u> <u>DYWGQGTLVTVSS</u>
53	OX40(17A9) VL	SSELTQDPAVSVALGQTVRITCQGDLSRSYYASWYQ QKPGQAPVLVIY <u>GKNNRPSGIPDRFSGSSSGNTASLTI</u> TGAQAEDEADYYC <u>NSRVMPHNRVFGGGTKLTV</u>
54	Fc knob chain of CD134-0093	Table 1
55	Fc hole chain of CD134-0093	Table 1
56	Fc knob chain of CD134-0094	Table 2
57	Fc hole chain of CD134-0094	Table 2
58	Fc knob chain of P1AE0085	Table 3
59	Fc hole chain of P1AE0085	Table 3
60	Fc knob chain of P1AE0086	Table 4
61	Fc hole chain of P1AE0086	Table 4
62	Fc knob chain of P1AE0087	Table 5
63	Fc hole chain of P1AE0087	Table 5
64	Fc knob chain of P1AE0839	Table 6
65	Fc hole chain of P1AE0839	Table 6
66	Fc knob chain of P1AE0821	Table 7
67	Fc hole chain of P1AE0821	Table 7
68	(49B4) VHCH1 Fc knob VH (28H1) (heavy chain 1)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGGGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDVAVYYCAREYYRGPYDY WGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEV QLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVR QAPGKGLEWVSAIWASGEQYYADSVKGRFTISRDN

SEQ ID NO:	Name	Sequence
		KNTLYLQMNSLRAEDTAVYYCAKGWLGNFYWGQ GTLVTVSS
69	(49B4) VHCH1 Fc hole VL (28H1) (heavy chain 2)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSED TAVYYCAREYYRGPYDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALGAPIEKTISKAKGQPREPQVCTLPSSRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPV LDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEIV LTQSPGTLSPGERATLSCRASQSVSRSLAWYQQK PGQAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQQGQVIPPTFGQGTKVEIK
70	(49B4) VLCL-light chain	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQ QKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLT SSLQPDDEATYYCQYSSQPYTFGQGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC
71	(49B4) VHCH1 Fc knob VH (4B9) (heavy chain 1)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSED TAVYYCAREYYRGPYDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPPV LDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEV QLLESGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAIIGSGASTYYADSVKGRFTISRDN KNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWGQ GTLVTVSS
72	(49B4) VHCH1 Fc hole VL (4B9) (heavy chain 2)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSED TAVYYCAREYYRGPYDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALGAPIEKTISKAKGQPREPQVCTLPSSRDELTKN

SEQ ID NO:	Name	Sequence
		QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPPV LSDSGSFLLVSKLTVDKSRWQQGNVFSCSVMEALH NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEIV LTQSPGTLSPGERATLSCRASQSVTSSYLAWYQQK PGQAPRLINVGSRRTATGIPDRFSGSGSGTDFTLTISR LEPEDFAVYYCQQGIMLPPTFGQGTKVEIK
73	(49B4) VHCH1 Fc knob VH (DP47) (heavy chain 1)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDVAVYYCAREYYRGPYDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPPV LSDSGSFLLVSKLTVDKSRWQQGNVFSCSVMEALH NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEIV QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSVMR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDN KNTLYLQMNSLRAEDTAVYYCAKSGFDYWGQGT LTVSS
74	(49B4) VHCH1 Fc hole VL (DP47) (heavy chain 2)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDVAVYYCAREYYRGPYDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALGAPIEKTISKAKGQPREPQVCTLPSPRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPPV LSDSGSFLLVSKLTVDKSRWQQGNVFSCSVMEALH NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEIV LTQSPGTLSPGERATLSCRASQSVSSYLAWYQQK PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISR LEPEDFAVYYCQQYGSSPLTFGQGTKVEIK
75	HC 1 (49B4) VHCH1_VHCH1 Fc knob VH (4B9)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITA DKSTSTAYMELSSLRSEDVAVYYCAREYYRGPYDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDGGGSGGGGSQVQLVQSGAEVKKPGSSVKV SCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGT ANYAQKFQGRVTITADKSTSTAYMELSSLRSEDVAV YYCAREYYRGPYDYWGQGTITVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV

SEQ ID NO:	Name	Sequence
		NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGP SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREP QVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGG GGSGGGGSGGGGSEVQLLESGLVQPGGSLRLSCA ASGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTY YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYY CAKGWFGGFNYWGQGLVTVSS
76	HC 2 (49B4) VHCH1_VHCH1 Fc hole VL (4B9)	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITTA DKSTSTAYMELSSLRSED TAVYYCAREYYRGPYDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDGGGGSGGGGSQVQLVQSGAEVKKPGSSVKV SCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGT ANYAQKFQGRVTITADKSTSTAYMELSSLRSED TAV YYCAREYYRGPYDYWGQGTITVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGP SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREP QVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWES NGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGG GSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRA SQSVTSSYLAWYQQKPGQAPRLLINVGSRRA TGIPTDR FSGSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFG QGTKVEIK
77	Peptide linker G4S	GGGGS
78	Peptide linker (G4S) ₂	GGGGS GGGGS
79	Peptide linker (SG4) ₂	SGGGGS GGGG
80	Peptide linker	GGGGGS GGGGSSGGGGS
81	Peptide linker (G4S) ₃	GGGGS GGGGSSGGGGS
82	Peptide linker G4(SG4) ₂	GGGGS GGGGSSGGG
83	Peptide linker (G4S) ₄	GGGGS GGGGSSGGGSSGGGGS
84	Peptide linker	GGGGS GGGGSSGGGSSGGGGS
85	Peptide linker	GSPGSSSSGS
86	Peptide linker	GSGSGSGS
87	Peptide linker	GSGSGNGS
88	Peptide linker	GGSGSGSG
89	Peptide linker	GGSGSG
90	Peptide linker	GGSG
91	Peptide linker	GGSGNGSG
92	Peptide linker	GGNGSGSG

SEQ ID NO:	Name	Sequence
93	Peptide linker	GGNGSG
94	IgG CH1 domain	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKV
95	IgG CH2 domain	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQESTYRWSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
96	IgG CH3 domain	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
97	human FAP	Uniprot accession no. Q12884
98	mouse FAP	UniProt accession no. P97321
99	CH1 connector	EPKSC
100	hinge	DKTHTCPXCP with X being S or P
101	hinge	HTCPXCP with X being S or P
102	hinge	CPXCP with X being S or P
103	IgG1, caucasian allotype	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL PGK
104	IgG1, afroamerican allotype	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL PGK
105	IgG2	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSN FGTQTYTCNVNHNKPSNTKVDKTKVERKCCVECPCPA PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI SVEWESNGQPENNYKTPPMLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
106	IgG3	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYTCNVNHNKPSNTKVDKRVELKTPLGDTTHT CPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKS CDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRT

SEQ ID NO:	Name	Sequence
		EVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPR EEQYNSTFRVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESSGQPENNYNTTPMMLDS GSFFLYSKLTVDKSRWQQGNIFSCSVMEALHNHRT QKSLSLSPGK
107	IgG4	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS LGTKITYTCNVDHKPSNTKVDKRVESKYGPPCPSCPA PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD KSRWQEGNVFSCSVMEALHNHNTQKSLSLSLGK
108	human OX40	UniProt no. P43489
109	human 4-1BB	UniProt no. Q07011
110	human CD27	UniProt no. P26842
111	human HVEM	UniProt no. Q92956
112	human CD30	UniProt no. P28908
113	human GITR	UniProt no. Q9Y5U5
114	murine OX40	UniProt no. P47741
115	human CD40	UniProt no. P25942
116	Fc knob chain of P1AE1122	See Table 8
117	Fc hole chain of P1AE1122	See Table 8
118	Fc knob chain of P1AE1942	See Table 9
119	Fc hole chain of P1AE1942	See Table 9
120	Fc knob chain of P1AE1887	See Table 10
121	Fc hole chain of P1AE1887	See Table 10
122	Fc knob chain of P1AE1888	See Table 11
123	Fc hole chain of P1AE1888	See Table 11
124	Fc knob chain of P1AE2254	See Table 12
125	Fc hole chain of P1AE2254	See Table 12
126	Fc knob chain of P1AE2340	See Table 13
127	Fc hole chain of P1AE2340	See Table 13

SEQ ID NO:	Name	Sequence
128	Light chain of P1AE2340	See Table 13
129	Fc knob chain of P1AE2735	See Table 14
130	Fc hole chain of P1AE2735	See Table 14
131	Fc knob chain of P1AE2743	See Table 15
132	Fc hole chain of P1AE2743	See Table 15
133	Fc knob chain of P1AE2762	See Table 16
134	Fc hole chain of P1AE2762	See Table 16
135	4-1BB (20H4.9) CDR-H1	GYYSWS
136	4-1BB (20H4.9) CDR-H2	EINHGGYVTYNPSLES
137	4-1BB (20H4.9) CDR-H3	DYGPGNYDWYFDL
138	4-1BB (20H4.9) CDR-L1	RASQSVSSYLA
139	4-1BB (20H4.9) CDR-L2	DASNRAT
140	4-1BB (20H4.9) CDR-L3	QQRSNWPPALT
141	4-1BB (20H4.9) VH	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWS WIRQSPEKGLEWIGEINHGGYVTYNPSLESRTISVD TSKNQFSLKLSSVTAADTAVYYCARDYGPNGYDWY FDLWGRGTLTVSS
142	4-1BB (20H4.9) VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQ QKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTI SSLEPEDFAVYYCQQRSNWPPALTFGGGTKVEIK
143	Fc knob chain of P1AE1899	See Table 26
144	Fc hole chain of P1AE1899	See Table 26
145	Fc knob chain of P1AE2051	See Table 27
146	Fc hole chain of P1AE2051	See Table 27
147	hu CD40 CDR-H1	GYIIH
148	hu CD40 CDR-H2	RVIPNAGGTSYNQKFKG
149	hu CD40 CDR-H3	EGIYW
150	hu CD40 CDR-L1	RSSQSLVHSNGNTFLH
151	hu CD40 CDR-L2	TVSNRFS
152	hu CD40 CDR-L3	SQTTHVPWT
153	hu CD40 VH	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYIIHW VRQAPGKGLEWVARVIPNAGGTSYNQKFKGRFTLSV DNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWGQ GTLTVSS
154	hu CD40 VL	DIQMTQSPSSLSASVGDRTTITCRSSQSLVHSNGNTFL HWYQQKPGKAPKLLIYTVSNRFSGVPSRFSGSGSGT DFLTISLQPEDFATYFCSQTTHVPWTFGQGTKVEIK

SEQ ID NO:	Name	Sequence
155	Fc knob chain of P1AE1799	See Table 32
156	Fc hole chain of P1AE1799	See Table 32
157	Fc knob chain of P1AE1902	See Table 33
158	Fc hole chain of P1AE1902	See Table 33
159	Fc knob chain of P1AE1800	See Table 34
160	Fc hole chain of P1AE1800	See Table 34
161	Fc knob chain of P1AE2052	See Table 35
162	Fc hole chain of P1AE2052	See Table 35
163	Fc knob chain of P1AE1901	See Table 36
164	Fc hole chain of P1AE1901	See Table 36
165	Fc knob chain of P1AE2255	See Table 37
166	Fc hole chain of P1AE2255	See Table 37
167	VH1a (CD40)	QVQLVQSGAEVKKPGASVKVSCKASGYSTFTGYYIH WVRQAPGQSLEWMGRVIPNAGGTSYNQKFKGRVTL TVDKSISTAYMELSRRLRSDDTAVYYCAREGIYWWG QGTTVTVSS
168	VH1b (CD40)	QVQLVQSGAEVKKPGASVKVSCKASGYSTFTGYYIH WVRQAPGKSLEWMGRVIPNAGGTSYNQKFKGRVTL TVDKSISTAYMELSRRLRSDDTAVYYCAREGIYWWG QGTTVTVSS
169	VH1c (CD40)	QVQLVQSGAEVKKPGASVKVSCKASGYSTFTGYYIH WVRQAPGQSLEWMGRVIPNAGGTSYNQKFKGRVTL TVDKSISTAYMELSRRLRSDDTAVYYCAREGIYWWG HGTTVTVSS
170	VH1d (CD40)	QVQLVQSGAEVKKPGASVKVSCKASGYSTFTGYYIH WVRQAPGQSLEWMGRVIPNAGGTSYNQKFKGRVTL SVDKSISTAYMELSRRLRSDDTAVYYCAREGIYWWG GTTVTVSS
171	VL1a (CD40)	DIVMTQTPLSLSVTPGQPASISCRSSQSLVHSNGNTFL HWYLQKPGQSPQLLIYTVSNRFGVDPDRFSGSGSGTD FTLKISRVEAEDVGVYFCSQTTHVPWTFGGGTKEIK
172	VL1b (CD40)	DIVVTQTPLSLSVTPGQPASISCRSSQSLVHSNGNTFL HWYLQKPGQSPQLLIYTVSNRFGVDPDRFSGSGSGTD FTLKISRVEAEDVGVYFCSQTTHVPWTFGGGTKEIK
173	VL1c (CD40)	DVVVTQTPLSLSVTPGQPASISCRSSQSLVHSNGNTFL HWYLQKPGQSPQLLIYTVSNRFGVDPDRFSGSGSGTD

SEQ ID NO:	Name	Sequence
		FTLKISRVEAEDVGVYFCSQTTHVPWTFGGGTKVEIK
174	VL1d (CD40)	DVVVTQTPLSLSVTPGQPASISCRSSQSLVHSNGNTFL HWYLLQKPGQSPQLLIYTVSNRFSGVPSRFSGSGSGTD FTLKISRVEAEDVGVYFCSQTTHVPWTFGGGTKLEIK
175	VH2a (CD40)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHW VRQAPGKGLEWVGRVIPNAGGTSYNQKFKGRFTISV DNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWGQ GTTVTVSS
176	VH2b (CD40)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHW VRQAPGKSLEWVGRVIPNAGGTSYNQKFKGRFTISV DNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWGQ GTTVTVSS
177	VH2c (CD40)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHW VRQAPGKGLEWVGRVIPNAGGTSYNQKFKGRFTISV DNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWGH GTTVTVSS
178	VH2d (CD40)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHW VRQAPGKGLEWVGRVIPNAGGTSYGDVSKGRFTISV DNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWGQ GTTVTVSS
179	VH2ab (CD40)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYMH WVRQAPGKGLEWVGRVIPNAGGTSYNQKFKGRFTIS VDNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWG QGTTVTVSS
180	VH2ac (CD40)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHW VRQAPGKGLEWVGRVIPNAGGTSYNQKVKGRFTISV DNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWGQ GTTVTVSS
181	VL2a (CD40)	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSNGNTFL HWYQQKPGKAPKLLIYTVSNRFSGVPSRFSGSGSGT DFLTISLQPEDFATYFCSQTTHVPWTFGGGTKVEIK
182	VL2b (CD40)	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSNGNTFL HWYQQKPGQSPKLLIYTVSNRFSGVPSRFSGSGSGTD FTLTISLQPEDFATYFCSQTTHVPWTFGGGTKVEIK
183	VL2ab (CD40)	DIQMTQSPSSLSASVGDRVTITCRASQSLVHSNGNTF LHWYQQKPGKAPKLLIYTVSNRFSGVPSRFSGSGSGT DFLTISLQPEDFATYFCSQTTHVPWTFGGGTKVEIK
184	VL2ac (CD40)	DIQMTQSPSSLSASVGDRVTITCRSSQSIVHSNGNTFL HWYQQKPGKAPKLLIYTVSNRFSGVPSRFSGSGSGT DFLTISLQPEDFATYFCSQTTHVPWTFGGGTKVEIK

General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

- 5 Amino acids of antibody chains are numbered and referred to according to the EU numbering systems according to Kabat (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest,

5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)) as defined above.

5

EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Recombinant DNA techniques

Standard methods were used to manipulate DNA as described in Sambrook et al.,
10 Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturer's instructions. General information regarding the nucleotide sequences of human immunoglobulin light and heavy chains is given in: Kabat, E.A. et al., (1991) Sequences of Proteins of Immunological Interest, Fifth Ed., NIH Publication No 91-3242.

15 DNA sequencing

DNA sequences were determined by double strand sequencing.

Gene synthesis

Desired gene segments were prepared by chemical synthesis at Geneart AG (Regensburg, Germany) from synthetic oligonucleotides by automated gene synthesis. The synthesized gene
20 fragments were cloned into an E. coli plasmid for propagation/amplification. The DNA sequences of subcloned gene fragments were verified by DNA sequencing. Alternatively, short synthetic DNA fragments were assembled by annealing chemically synthesized oligonucleotides or via PCR. The respective oligonucleotides were prepared by metabion GmbH (Planegg-Martinsried, Germany).

25 Cell culture techniques

Standard cell culture techniques were used as described in Current Protocols in Cell Biology (2000), Bonifacino, J.S., Dasso, M., Harford, J.B., Lippincott-Schwartz, J. and Yamada, K.M. (eds.), John Wiley & Sons, Inc.

Reagents

All commercial chemicals, antibodies and kits were used as provided according to the manufacturer's protocol if not stated otherwise.

Example 1

5 Generation of bispecific contorsbodies

1.1 Construction of the expression plasmids for the bispecific contorsbodies

For the expression of bispecific contorsbodies as reported herein a transcription unit comprising the following functional elements was used:

- 10 - the immediate early enhancer and promoter from the human cytomegalovirus (P-CMV) including intron A,
- a human heavy chain immunoglobulin 5'-untranslated region (5'UTR),
 - a murine immunoglobulin heavy chain signal sequence,
 - a nucleic acid encoding the respective circular fusion polypeptide, and
 - the bovine growth hormone polyadenylation sequence (BGH pA).

15 Beside the expression unit/cassette including the desired gene to be expressed the
basic/standard mammalian expression plasmid contains

- an origin of replication from the vector pUC18 which allows replication of this plasmid in *E. coli*, and
- a beta-lactamase gene which confers ampicillin resistance in *E. coli*.

20 **1.2 Expression of the bispecific contorsbodies**

Transient expression of the bispecificantigen binding molecules was performed in suspension-adapted HEK293F (FreeStyle 293-F cells; Invitrogen) cells with Transfection Reagent 293-free (Novagen).

Cells were passaged, by dilution, at least four times (volume 30 ml) after thawing in a 125
25 ml shake flask (Incubate/Shake at 37 °C, 7 % CO₂, 85 % humidity, 135 rpm). The cells were
expanded to 3x10⁵ cells/ml in 250 ml volume. Three days later, cells were split and new seeded
with a density of 7*10⁵ cells/ml in a 250 ml volume in a 1 liter shake flask. Transfection will be
24 hours later at a cell density around 1.4 - 2.0x10⁶ cells/ml.

Before transfection 250 µg plasmid-DNA were diluted in a final volume of 10 ml with pre-heated (water bath; 37 °C) Opti-MEM (Gibco). The solution was gently mixed and incubated at room temperature for not longer than 5 min. Then 333.3 µl of the 293-free transfection reagent were added to the DNA-OptiMEM-solution. Thereafter the solution was gently mixed and
5 incubated at room temperature for 15-20 minutes. The whole volume of mixture was added to 1 L shake flask with 250 ml HEK-cell-culture-volume.

Incubate/Shake at 37 °C, 7 % CO₂, 85 % humidity, 135 rpm for 6 or 7 days.

The supernatant was harvested by a first centrifugation-step at 2,000 rpm, 4 °C, for 10 minutes. Then the supernatant was transferred into a new centrifugation-flask for a second
10 centrifuge at 4,000 rpm, 4 °C, for 20 minutes. Thereafter the cell-free-supernatant was filtered through a 0.22 µm bottle-top-filter and stored in a freezer (-20 °C).

1.3 Purification of the bispecific contorsbodies

The antigen binding molecule-containing culture supernatants were filtered and purified by two chromatographic steps. The antibodies were captured by affinity chromatography using
15 HiTrap MabSelectSuRe (GE Healthcare) equilibrated with PBS (1 mM KH₂PO₄, 10 mM Na₂HPO₄, 137 mM NaCl, 2.7 mM KCl), pH 7.4. Unbound proteins were removed by washing with equilibration buffer, and the antigen binding molecule was recovered with 50 mM citrate buffer, pH 2.8, and immediately after elution neutralized to pH 6.0 with 1 M Tris-base, pH 9.0. Size exclusion chromatography on Superdex 200TM (GE Healthcare) was used as second
20 purification step. The size exclusion chromatography was performed in 20 mM histidine buffer, 0.14 M NaCl, pH 6.0. The bispecificantigen binding molecules containing solutions were concentrated with an Ultrafree -CL centrifugal filter unit equipped with a Biomax-SK membrane (Millipore, Billerica, MA) and stored at -80 °C.

1.4 Mass spectrometric analysis of the bispecific contorsbodies

25 PNGase F was obtained from Roche Diagnostics GmbH (14.3 U / µl; solution in sodium phosphate, EDTA and glycerol). A protease specifically cleaving in the hinge region of an IgG antibody was freshly reconstituted from a lyophilisate prior to digestion.

Enzymatic deglycosylation of with PNGase F

50 µg of antigen binding molecule was diluted to a final concentration of 0.6 mg/ml with
30 10 mM sodium phosphate buffer, pH 7.1, and deglycosylated with 1µl PNGase F at 37°C for 16 hours.

Enzymatic cleavage

The deglycosylated sample was diluted to a final concentration of 0.5 mg/ml with 200 mM Tris buffer, pH 8.0, and subsequently digested with the IgG specific protease at 37°C for 1 hour.

ESI-QTOF mass spectrometry

The sample was desalted by HPLC on a Sephadex G25 column (Kronlab, 5x250mm, TAC05/250G0-SR) using 40% acetonitrile with 2% formic acid (v/v). The total mass was determined via ESI-QTOF MS on the maXis 4G UHR-QTOF MS system (Bruker Daltonik) equipped with a TriVersa NanoMate source (Advion). Calibration was performed with sodium iodide (Waters ToF G2-Sample Kit 2 Part: 700008892-1). For the digested antigen binding molecule, data acquisition was done at 1000-4000 m/z (ISCID: 30 eV). The raw mass spectra were evaluated and transformed into individual relative molar masses. For visualization of the results proprietary software was used to generate deconvoluted mass spectra.

Example 2

Preparation of bispecific antibodies with two antigen binding domains binding to OX40 and one antigen binding domain binding to FAP (FAP-OX40 contorsbodies)

The generation and preparation of the FAP binders is described in WO 2012/020006 A2, which is incorporated herein by reference. The OX40 binder is described in WO 2017/055398 A2.

2.1 Preparation of FAP (4B9)-OX40 (49B4) contorsbody CD134-0093

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in

Figure 1A:

- first fusion polypeptide (from N- to C-terminus): VH (OX40)-CH1, (G4S)2 connector , IgG1 hinge, Fc knob, (G4S)2 connector, VL(OX40)-Ckappa, (G4S)2 connector, VH(FAP)-Ckappa
- second fusion polypeptide (from N- to C-terminus): VH (OX40)-CH1, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(OX40)-Ckappa, (G4S)2 connector, VL(FAP)-CH1.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 1 shows the amino acid sequences of the bispecific antibody CD134-0093.

Table 1: Sequences of CD134-0093

SEQ ID NO:	Description	Sequence
54	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSTLSA SVGDRVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQYSSQPY TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSS TLTLISKADYEEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGGSEVQLLESGGGLVQPGLSLRLSCAASGFTSSYA MSWVRQAPGKGLEWVSAIIGSGASTYYADSVKGRFTISR NSKNTLYLQMNSLRAEDTAVYYCAKGFVGGFNYWGQGT LTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDYSLSS TLTLISKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC
55	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSTLSA SVGDRVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQYSSQPY TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSS TLTLISKADYEEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVTSSYLA WYQQKPGQAPRLLINVGSRRTGIPDRFSGSGSGTDFTLT SRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIKSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT S GVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSC

A schematic scheme of the assembled structure is shown in **Figure 1E**.

2.2 Preparation of FAP (4B9)-OX40 (49B4) contorsbody CD134-0094

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in

Figure 1B:

- 5 - first fusion polypeptide (from N- to C-terminus): VH (OX40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(OX40)-CH1, (G4S)2 connector, VL(FAP)-Ckappa
- second fusion polypeptide (from N- to C-terminus): VH (OX40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(OX40)-CH1, (G4S)2 connector, VH(FAP)-CH1.

10 The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

15 Table 2 shows the amino acid sequences of the bispecific antibody CD134-0094.

Table 2: Sequences of CD134-0094

SEQ ID NO:	Description	Sequence
56	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA SVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGECDDGGGSGGGGSDKT HTCPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGKGGGSGGGGSDIQMT QSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKL LIYDASSLESQVPSRFSGSGSGTEFTLTISLQPDDEFATYYC QQYSSQPYTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVT SSVLAWYQQKPGQAPRLINVGSRRTATGIPDRFSGSGSGTD FTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC

57	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA SVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGECGGGGSGGGGSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQ SPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLL IYDASSLESQVPSRFSGSGSGTEFTLTISSLQPDDEFATYYCQ QYSSQPYTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC GGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFS SYAMSWVRQAPGKGLEWVSAIGSGASTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSC
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A schematic scheme of the assembled structure is shown in **Figure 1F**.

2.3 Preparation of FAP (28H1)-OX40 (49B4) contorsbody P1AE0085

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in

5 **Figure 1A:**

- first fusion polypeptide (from N- to C-terminus): VH (OX40)-CH1, (G4S)₂ connector, IgG1 hinge, Fc knob, (G4S)₂ connector, VL(OX40)-Ckappa, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa
- second fusion polypeptide (from N- to C-terminus): VH (OX40)-CH1, (G4S)₂ connector,
- 10 IgG1 hinge, Fc hole, (G4S)₂ connector, VL(OX40)-Ckappa, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The

15 knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 3 shows the amino acid sequences of the bispecific antibody P1AE0085.

Table 3: Sequences of P1AE0085

SEQ ID NO:	Description	Sequence
58	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDYAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSTLSA SVGDRVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYSSQPY TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAA SGFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLG FDYWGQGTITVTVSSASVAAPSVFIFPPSDEQLKSGTASVVC LLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C
59	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDYAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSTLSA SVGDRVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYSSQPY TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRA SQSVSRSYLAWYQQKPGQAPRLLIIGASTRATGIPDRFSGS GSGTDFLTISRLEPEDFAVYYCQQGQVIPPTFGQGTKVEIK SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSC

A schematic scheme of the assembled structure is shown in **Figure 1G**.

2.4 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE0086

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in **Figure 1A**:

- 5 - first fusion polypeptide (from N- to C-terminus): VH (OX40)-CH1, (G4S)₂ connector, IgG1 hinge, Fc knob, (G4S)₂ connector, VL(OX40)-Ckappa, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa
- second fusion polypeptide (from N- to C-terminus): VH (OX40)-CH1, (G4S)₂ connector, IgG1 hinge, Fc hole, (G4S)₂ connector, VL(OX40)-Ckappa, GGGGSGGGGSGGGSGGGGS
- 10 (SEQ ID NO:84) connector, VL(FAP)-CH1.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V

15 mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 4 shows the amino acid sequences of the bispecific antibody P1AE0086 (Contorsbody 7).

Table 4: Sequences of P1AE0086

SEQ ID NO:	Description	Sequence
60	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHITCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSTLSA SVGDRVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQYSSQPY TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSLSS TLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGGSGGGSGGGGSEVQLLESGLLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWFGG

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		FNYWGQGTTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSITY SLSSSTLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C
61	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGGSFLLVSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSTLSA SVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYSSQPY TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSITYSLSS TLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GGGGGGSGGGGGGGSEIVLTQSPGTLSLSPGERATLSCRA SQSVTSSYLAWYQQKPGQAPRLLINVGSRRTGIPDRFSGS GSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIK SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSC

A schematic scheme of the assembled structure is shown in **Figure 1G**.

2.5 Preparation of FAP (28H1)-OX40 (49B4) contorsbody P1AE0087

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in

5 **Figure 1B:**

- first fusion polypeptide (from N- to C-terminus): VH (OX40)-Ckappa, (G4S)₂ connector, IgG1 hinge, Fc knob, (G4S)₂ connector, VL(OX40)-CH1, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-Ckappa.
- second fusion polypeptide (from N- to C-terminus): VH (OX40)-Ckappa, (G4S)₂ connector, IgG1 hinge, Fc hole, (G4S)₂ connector, VL(OX40)-CH1, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-CH1.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in

the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 5 shows the amino acid sequences of the bispecific antibody P1AE0087.

Table 5: Sequences of P1AE0087

SEQ ID NO:	Description	Sequence
62	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA SVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGECDDGGGSGGGGSDKT HTCPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMT QSPSTLSASVGDRVITTCRASQSISSWLAWYQQKPGKAPKL LIYDASSLESQVPSRFSGSGSGTEFTLTISSLQPDDEFATYYC QQYSSQPYTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATL SCRASQSVSRSYLAWYQQKPGQAPRLLIIGASTRATGIPDR FSGSGSGTDFTLTISRLEPEDFAVYYCQQGQVIPPFTFGQGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKSTYSLSSLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC
63	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA SVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGECDDGGGSGGGGSDKT HTCPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQ SPSTLSASVGDRVITTCRASQSISSWLAWYQQKPGKAPKLL IYDASSLESQVPSRFSGSGSGTEFTLTISSLQPDDEFATYYCQ QYSSQPYTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YLSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC GGGGSGGGGSGGGGSGGGGSEVQLLESGLVQPGGSLRL SCAASGFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYY ADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKG

		WLGNFYDWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
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A schematic scheme of the assembled structure is shown in **Figure 1H**.

2.6 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE0839

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in

5 **Figure 1B:**

- first fusion polypeptide (from N- to C-terminus): VH (OX40)-Ckappa, (G4S)₂ connector, IgG1 hinge, Fc knob, (G4S)₂ connector, VL(OX40)-CH1, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-Ckappa.

10 - second fusion polypeptide (from N- to C-terminus): VH (OX40)-Ckappa, (G4S)₂ connector, IgG1 hinge, Fc hole, (G4S)₂ connector, VL(OX40)-CH1, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-CH1.

15 The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 6 shows the amino acid sequences of the bispecific antibody P1AE0839.

Table 6: Sequences of P1AE0839

SEQ ID NO:	Description	Sequence
64	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA SVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGECDDGGGSGGGGSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNV SCSVMEALHNHYTQKSLSLSPGKGGGSGGGGSDIQMT QSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPKAPKL LIYDASSLESQVPSRFGSGSGTEFTLTISSLQPDDFATYYC QQYSSQPYTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTSGG

		TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CGGGSGGGSGGGSGGGSGGGSEIVLTQSPGTLSPGERATL SCRASQSVTSSYLAWYQQKPGQAPRLINVGSRRTATGIPDR FSGSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC
65	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAIVYYCAREYYRGPYDYWGQGTITVTVSSA SVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHK KVYACEVTHQGLSSPVTKSFNRGECDDGGGSGGGGSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSDIQTQ SPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLL IYDASSLESQVPSRFSGSGSGTEFTLTISSLQPDFFATYYCQ QYSSQPYTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC GGGGSGGGSGGGSGGGGSEVQLLESGLVQPGGSLRL SCAASGFTFSYAMSWVRQAPGKGLEWVSAIIGSGASTYY ADSVKGRFTISRDNKNTLYLQMNSLRAEDTAIVYYCAKG WFGGFNYWGQGLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

A schematic scheme of the assembled structure is shown in **Figure 1H**.

2.7 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE0821

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in

5 **Figure 1C:**

- first fusion polypeptide (from N- to C-terminus): VL (OX40)-Ckappa, (G4S)² connector, IgG1 hinge, Fc knob, (G4S)² connector, VH(OX40)-CH1, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.
- second fusion polypeptide (from N- to C-terminus): VL (OX40)-Ckappa, (G4S)² connector, IgG1 hinge, Fc hole, (G4S)² connector, VH(OX40)-CH1, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

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The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors

according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

- 5 Table 7 shows the amino acid sequences of the bispecific antibody P1AE0821 (Contorsbody 11).

Table 7: Sequences of P1AE0821

SEQ ID NO:	Description	Sequence
66	first fusion polypeptide (Fc knob)	DIQMTQSPSTLSASVGDRVTTTCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPDFA TYYCQQYSSQPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTK NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSPGKGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSC KASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQ KFQGRVTTITADKSTSTAYMELSSLRSEDTAVYYCAREYYR GPYDYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGG GSGGGGSGGGSGGGGSEVQLLESGLVQPGGSLRLSCA ASGFTFSSYAMSWVRQAPGKGLEWVSAIGSGASTYYADS VKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKGWFG GFNYWGQGTITVTVSSASVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
67	second fusion polypeptide (Fc hole)	DIQMTQSPSTLSASVGDRVTTTCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPDFA TYYCQQYSSQPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALGAPIEKTISKAKGQPREPQVCTLPSPRDELTK NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSPGKGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSC KASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQ KFQGRVTTITADKSTSTAYMELSSLRSEDTAVYYCAREYYR GPYDYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAA

		LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGG GSGGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSCR ASQSVTSSYLAWYQQKPGQAPRLINVGSRRTATGIPDRFSG SSGTDFLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEI KSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSC
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A schematic scheme of the assembled structure is shown in **Figure 11**.

2.8 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE1122 (Contorsbody 1)

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in

5 **Figure 1D:**

- first fusion polypeptide (from N- to C-terminus): VH (OX40)-CH1, (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(OX40)-Ckappa, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

10 - second fusion polypeptide (from N- to C-terminus): VH (OX40)-CH1, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(OX40)-Ckappa, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The
 15 knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 8 shows the amino acid sequences of the bispecific antibody P1AE1122.

Table 8: Sequences of P1AE1122:

SEQ ID NO:	Description	Sequence
116	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDVAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHCTPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN

		YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH ALHNHYTQKSLSLSPGKGGGGSGGGSDIQMTQSPSTLSA SVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISLQPDFFATYYCQQYSSQPY TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSCRA SQSVTSSYLAWYQQKPGQAPRLLINVGSRRAATGIPDRFSGS GSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIK SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSC
117	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMH ALHNHYTQKSLSLSPGKGGGGSGGGSDIQMTQSPSTLSA SVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISLQPDFFATYYCQQYSSQPY TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGSGGGSGGGGSEVQLLESGLLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGFVGG FNYWGQGTITVTVSSASVAAPSVFIFPPSDEQLKSGTASVVC LLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKDY SLSSLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C

A schematic scheme of the assembled structure is shown in **Figure 1J**.

2.9 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE1942 (Contorsbody 2)

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- 5 - first fusion polypeptide (from N- to C-terminus): VL (OX40)-Ckappa, (G4S)₂ connector, IgG1 hinge, Fc knob, (G4S)₂ connector, VH(OX40)-CH1, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.
- second fusion polypeptide (from N- to C-terminus): VL (OX40)-Ckappa, (G4S)₂ connector, IgG1 hinge, Fc hole, (G4S)₂ connector, VH(OX40)-CH1,
- 10 GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 9 shows the amino acid sequences of the bispecific antibody P1AE1942.

Table 9: Sequences of P1AE1942:

SEQ ID NO:	Description	Sequence
118	first fusion polypeptide (Fc knob)	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISLQPDFA TYQCQQYSSQPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTK NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHLEALHNHYTQK SLSPGKGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSC KASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQ KFQGRVTTITADKSTSTAYMELSSLRSED TAVYYCAREYYR GPYDYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVPSSSLGTQTYICNVNHKPSNTKVDDKKVEPKSCGG GSGGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSCR ASQSVTSSYLAWYQQKPGQAPRLINVGSRRTATGIPDRFSG SGGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEI KSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPSSSLGTQ TYICNVNHKPSNTKVDDKKVEPKSC
119	second fusion polypeptide (Fc hole)	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISLQPDFA TYQCQQYSSQPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALGAPIEKTISKAKGQPREPQVCTLPSPRDELTK NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMHLEALHNHYTQK SLSPGKGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSC KASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQ KFQGRVTTITADKSTSTAYMELSSLRSED TAVYYCAREYYR

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		GPYDYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGG GSGGGGSGGGSGGGGSEVQLLESQGGGLVQPGGSLRLSCA ASGFTFSSYAMSWVRQAPGKGLEWVSAHGSASTYYADS VKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKGWFG GFNYWGQGTLLTVSSASVAAPSVFIFPPSDEQLKSGTASV CLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
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2.10 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE1887 (Contorsbody 3)

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- first fusion polypeptide (from N- to C-terminus): VL (OX40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VH(OX40)-CH1, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.

- second fusion polypeptide (from N- to C-terminus): VH (OX40)-CH1, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(OX40)-Ckappa, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

- 10 The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V
- 15 mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 10 shows the amino acid sequences of the bispecific antibody P1AE1887.

Table 10: Sequences of P1AE1887:

SEQ ID NO:	Description	Sequence
120	first fusion polypeptide (Fc knob)	DIQMTQSPSTLSASVGDRVITTCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPDFA TYQCQYSSQPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTK NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPPVLD DGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQK SLSLSPGKGGGGSGGGGSQVQLVQSGAEVKKPKGSSVKVSC

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		KASGGTFSSY AISWVRQAPGQGLEWMGGIPIFGTANYAQ KFQGRVTITADKSTSTAYMELSSLRSED TAVYYCAREYYR GPYDYWGQGT TTVTVSSASTKGPSVFPLAPSSKSTSGGTA LGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGG GGSGGGSGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSY AMSWVRQAPGKGLEWVSAIIGSGASTYYADS VKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGWFG GFNYWGQGT LTVTVSSASVAAPS VFIFPPSDEQLKSGTASV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
121	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKV SCKASGGTFSSY AISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSED TAVYYCAREYYRGPYDYWGQGT TTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTTPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSTLSA SVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISLQPDDFATYYCQQYSSQPY TFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GGGGGGSGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRA SQSVTSSYLAWYQQKPGQAPRLLINVGSRRA TGI PDRFSGS GSGTDFLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIK SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSC

A schematic scheme of the assembled structure is shown in **Figure 1K**.

2.11 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE1888 (Contorsbody 4)

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- 5 - first fusion polypeptide (from N- to C-terminus): VH(OX40)-CH1, (G4S)₂ connector, IgG1 hinge, Fc knob, (G4S)₂ connector, VL(OX40)-Ckappa, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.
- second fusion polypeptide (from N- to C-terminus): VL(OX40)-Ckappa, (G4S)₂ connector, IgG1 hinge, Fc hole, (G4S)₂ connector, VH(OX40)-CH1,
- 10 GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 11 shows the amino acid sequences of the bispecific antibody P1AE1888.

Table 11: Sequences of P1AE1888:

SEQ ID NO:	Description	Sequence
122	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSTLSA SVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSL ESGVPSRFSGSGSGTEFTLTISLQPDFAFYCYQQYSSQPY TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLN NFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSL SLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGGSGGGGSGGGGSEVQLLESGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGWFGG FNYWGQGTITVTVSSASVAAPSVFIFPPSDEQLKSGTASVVC LLN NFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C
123	second fusion polypeptide (Fc hole)	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESQVPSRFSGSGSGTEFTLTISLQPDFA FYCYQQYSSQPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCCLN NFYFPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYLSLSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALGAPIEKTISKAKGQPREPQVCTLPSPRDELTK NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD DGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGKGGGGSGGGGSGVQLVQSGAEVKKPGSSVKVSC KASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQ

		KFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCAREYYR GPYDYGWGGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGG GSGGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSCR ASQSVTSSYLAWYQQKPGQAPRLLINVGSRRTATGIPDRFSG SGSGTDFLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEI KSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSC
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2.12 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE2254 (Contorsbody 5)

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- first fusion polypeptide (from N- to C-terminus): VH(OX40)-CH1_EE (K147E, K213E),
 5 (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(OX40)-Ckappa_RK (E123R, Q124K), GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.
- second fusion polypeptide (from N- to C-terminus): VH(OX40)-CH1_EE (K147E, K213E), (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(OX40)-Ckappa_RK (E123R, Q124K), GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

- 10 The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V
- 15 mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)). Furthermore, in the CH and Ckappa fused to the VL and VH of OX40, respectively, amino acid mutations (so-called charged residues) were introduced to prevent the generation of Bence Jones proteins and to further facilitate the correct pairing, i.e negative charges in the CH1 domain (K147E, K213E, numbering according Kabat EU index) and positive charges in the CL domain
- 20 of the anti-OX40 binder 49B4 (E123R and Q124K, numbering according to Kabat EU index).

Table 12 shows the amino acid sequences of the bispecific antibody P1AE2254.

Table 12: Sequences of P1AE2254:

SEQ ID NO:	Description	Sequence
124	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDVAVYYCAREYYRGPYDYGWGGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAAALGCLVEDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN

		VNHKPSNTKVDEKVEPKSCGGGGSGGGGSDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTL PPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA LHNHYTQKSLSLSPGKGGGGSGGGGSDIQTQSPSTLSAS VGDRVTTICRASQSISSWLAWYQQKPGKAPKLLIYDASSLE SGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQYSSQPYT FGQGTKVEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGGSGGGSGGGGSEVQLLESGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGWFGG FNYWGQGT LTVVSSASVAAPSVFIFPPSDEQLKSGTASVVC LLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTY SLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C
125	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYA QKFQGRVTITADKSTSTAY MELSSLRSED TAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTWSN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDEKVEPKSCGGGGSGGGGSDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTL PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCFSVMHEA LHNHYTQKSLSLSPGKGGGGSGGGGSDIQTQSPSTLSAS VGDRVTTICRASQSISSWLAWYQQKPGKAPKLLIYDASSLE SGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQYSSQPYT FGQGTKVEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSGGGGSGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRA SQSVTSSYLAWYQQKPGQAPRLINVGSRRTATGIPDRFSGS GSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIK SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDDKKVEPKSC

A schematic scheme of the assembled structure is shown in **Figure 1L**.

2.13 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE2340 (Contorsbody 6)

A bispecific antibody comprising two fusion polypeptides and a light chain was cloned as follows:

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- first fusion polypeptide (from N- to C-terminus): VH(OX40)-CH1_EE (K147E, K213E), (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(OX40)-Ckappa_RK (E123R, Q124K), GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.
- second fusion polypeptide (from N- to C-terminus): VH(OX40)-CH1_EE (K147E, K213E), (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(OX40)-Ckappa_RK (E123R, Q124K).
- light chain: VL(FAP)-CH1.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)). Furthermore, the CH and Ckappa fused to the VL and VH of OX40, respectively, amino acid mutations (so-called charged residues) were introduced to prevent the generation of Bence Jones proteins and to further facilitate the correct pairing, i.e negative charges in the CH1 domain (K147E, K213E, numbering according Kabat EU index) and positive charges in the CL domain of the anti-OX40 binder 49B4 (E123R and Q124K, numbering according to Kabat EU index).

Table 13 shows the amino acid sequences of the bispecific antibody P1AE2340.

Table 13: Sequences of P1AE2340:

SEQ ID NO:	Description	Sequence
126	first fusion polypeptide (Fc knob)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDEKVEPKSCGGGSGGGGSDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTL PPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSTLSAS VGDRVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSLE SGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQYSSQPYT FGQGTKVEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLN FYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSST LTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGECGGG GSGGGGSGGGGSGGGGSEVQLLESGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTVYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWFGG

		FNYWGQGTTLTVSSASVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS STYSLSSSTLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
127	second fusion polypeptide (Fc hole)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSTLGTQTYICN VNHKPSNTKVDEKVEPKSCGGGSGGGGSDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTL PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGKGGGSGGGGSDIQMTQSPSTLSAS VGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLE SGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYSSQPYT FGQGTKVEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSVTEQDSKDS TYSLSSTLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
128	Light Chain	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKP GQAPRLINVGSRRTATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQGIMLPPTFGQGTKVEIKSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSTLGTQTYICNVNHKPSNTKVDEK KVEPKSC

A schematic scheme of the assembled structure is shown in **Figure 1M**.

2.14 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE2735 (Contorsbody 8)

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- 5 - first fusion polypeptide (from N- to C-terminus): VH(FAP)-Ckappa,
GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(OX40)-CH1, (G4S)2
connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(OX40)-Ckappa.
- 10 - second fusion polypeptide (from N- to C-terminus): VL(FAP)-CH1,
GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(OX40)-CH1, (G4S)2
connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(OX40)-Ckappa.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in

the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 14 shows the amino acid sequences of the bispecific antibody P1AE2735.

Table 14: Sequences of P1AE2735:

SEQ ID NO:	Description	Sequence
129	first fusion polypeptide (Fc knob)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA PGKGLEWVSAIIGSGASTYYADSVKGRFTISRDN SKNTLYL QMNSLRAEDTAVYYCAKGWFGGFNYWGQGLTVTVSSAS VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGECGGGGSGGGSGGGGS GGGGSQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAI SWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADKS TSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGT TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SLGTQTYICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTH TCPPEAAGGPPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SLTQVQLQDGLNGKEYKCKVSNKALGAPIEKISKAKGQPR EPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSDIQMTQ SPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLL IYDASSLESQVPSRFSGSGSGTEFTLTISSLQPDFFATYYCQ QYSSQPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK STYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
130	second fusion polypeptide (Fc hole)	EIVLTQSPGTLSPGERATLSCRASQSVTSSYLAWYQQK PQAPRLINVGSRRTATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQGIMLPPTFGQGTKVEIKSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPS SLGTQTYICNVNHKPSNTKVDK KVEPKSCGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKK PGSSVKVSCKASGGTFSSYAIWVRQAPGQGLEWMGGIPI FGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVY YCAREYYRGPYDYWGQGT TVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVTVPS SLGTQTYICNVNHKPSNTKVDK KVEPKSCGGGGSGGGGSDKTHTCPPEAAGGPPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTQVQLQDGLNGKEYKCKV SNKALGAPIEKISKAKGQPREPQVCTLPPSRDELTKNQV SLCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFF LVSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSL SPGKGGGGSGGGGSDIQMTQSPSTLSASVGDRVTITCRASQ SISSWLAWYQQKPGKAPKLLIYDASSLESQVPSRFSGSGSGT

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		EFTLTISLQPDDEFATYYCQQYSSQPYTFGQGTKVEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
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A schematic scheme of the assembled structure is shown in **Figure 1N**.

2.15 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE2743 (Contorsbody 9)

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- 5 - first fusion polypeptide (from N- to C-terminus): VH(FAP)-Ckappa, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(OX40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VH(OX40)-CH1.
- second fusion polypeptide (from N- to C-terminus): VL(FAP)-CH1, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(OX40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VH(OX40)-CH1.
- 10

- The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).
- 15

Table 15 shows the amino acid sequences of the bispecific antibody P1AE2743.

Table 15: Sequences of P1AE2743:

SEQ ID NO:	Description	Sequence
131	first fusion polypeptide (Fc knob)	EVQLLES GGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA PGKGLEWVSAIIGSGASTYYADSVKGRFTISRDN SKNTLYL QMNSLRAEDTAVYYCAKGWFGGFNYWGQGLTVTVSSAS VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHK KVYACEVTHQGLSSPVTKSFNRGECGGGGSGGGSGGGGS GGGGSDIQMTQSPSTLSASVGDRTITCRASQSISSWLAWY QQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISL QPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGECGGGGSGGGSGDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYCKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPC

		RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSQVQLVQSGAEVKKPGSS VKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTA NYAQKFQGRVTITADKSTSTAYMELSSLRSEDYAVYYCAR EYYRGPYDYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SC
132	second fusion polypeptide (Fc hole)	EIVLTQSPGTLSPGERATLSCRASQSVTSSYLAWYQQKP GQAPRLINVGSRRTATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQGIMLPPTFGQGTKEIKSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCGGGGSGGGSGGGSGGGSGGGSDIQTQSPSTLSAS VGDRVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSLE SGVPSRFSGSGSGTEFTLTISSLQPDDEYATYYCQYSSQPYT FGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSST LTLTKADYEKKHKVYACEVTHQGLSSPVTKSFNRGECGGG SGGGGSDKTHTCPPCPAPEAAGGSPVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK SRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGKGGGGSG GGGSQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTS TAYMELSSLRSEDYAVYYCAREYYRGPYDYWGQGTITVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSC

2.16 Preparation of FAP (4B9)-OX40 (49B4) contorsbody P1AE2762 (Contorsbody 10)

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- first fusion polypeptide (from N- to C-terminus): VH(FAP)-Ckappa,
- 5 GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(OX40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(OX40)-CH1.
- second fusion polypeptide (from N- to C-terminus): VH(FAP)-Ckappa,
- GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(OX40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(OX40)-CH1.

- 10 The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in

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the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 16 shows the amino acid sequences of the bispecific antibody P1AE2762.

Table 16: Sequences of P1AE2762:

SEQ ID NO:	Description	Sequence
133	first fusion polypeptide (Fc knob)	EIVLTQSPGTLSPGERATLSCRASQSVTSSYLAWYQQKP GQAPRLINVGSRRTGIPDRFSGSGSGTDFLTISRLEPEDF AVYYCQQGIMLPPTFGQGTKEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDYSLSSITLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGECGGGSGGGSGGGSGGGSGVQLVQSGA EVKKPGSSVKVSKASGGTFSSYAISWVRQAPQGQLEWM GGIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSED TAVYYCAREYYRGPYDYWGQGTITVTVSSASVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSSITLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGECGGGSGGGSGGGSDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNQKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLT PCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEAL HNHYTQKSLSLSPGKGGGSGGGSGGGSDIQMTQSPSTLSASV GDRVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES GVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQYSSQPYTF GQGTKEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTWNSGALTSGLVHTFPAVLQSSGLYSLSSVTVT PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
134	second fusion polypeptide (Fc hole)	EVQLLESQGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA PGKGLEWVSAIIGSGASTYYADSVKGRFTISRDNKNTLYL QMNSLRADDTAVYYCAKGWFGGFNYWGQGLTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNS GALTSGLVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCGGGSGGGSGGGSGGGSGQ VQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAP QGQLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAYM ELSSLRSEDTAVYYCAREYYRGPYDYWGQGTITVTVSSAS VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDYSLSSITLSKADYEKHK KVYACEVTHQGLSSPVTKSFNRGECGGGSGGGSGGGSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNQKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC CSVMHEALHNHYTQKSLSLSPGKGGGSGGGSGGGSDIQMTQ SPSTLSASVGDRTITTCRASQSISSWLAWYQQKPGKAPKLL IYDASSLESQVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQ

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		QYSSQPYTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
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2.17 Biochemical analysis of the molecules after purification

Table 17 summarizes the yield and final monomer content of the FAP OX40 contorsbodies.

Table 17 Biochemical analysis of the FAP OX40 contorsbodies

Construct	MW [kD]	Monomer [%] (SEC)	Yield [mg/l]
contorsbody CD134-0093	194.5	100	1.0
contorsbody CD134-0094	194.8	100	0.5
contorsbody P1AE0085	195.7	97.0	16.4
contorsbody P1AE0086	195.6	96.4	11.2
contorsbody P1AE0087	196.0	96.5	2.0
contorsbody P1AE0839		100	1.2
contorsbody P1AE0821		100	0.12
contorsbody P1AE1122		98.6	4.0
contorsbody P1AE1942		100	1.32
contorsbody P1AE1887		100	1.44
contorsbody P1AE1888		100	1.24
contorsbody P1AE2254		100	3.52
contorsbody P1AE2340		100	6.44
contorsbody P1AE2735		100	1.52
contorsbody P1AE2743		100	1.96
contorsbody P1AE2762		100	2.72

5

2.18 Preparation of bispecific OX40 antibodies as control molecules

As control the following bispecific anti-OX40 antibodies were prepared:

a) a bispecific antibody with bivalent binding for OX40 and monovalent binding for FAP was prepared in analogy with example 4.4 of WO 2017/055398 A2 (2+1 format). In this molecule, the first heavy chain (HC 1) was comprised of one Fab unit (VHCH1) of the anti-OX40 binder 49B4 followed by Fc knob chain fused by a (G4S) linker to a VH domain of the anti-FAP binder 28H1 or 4B9. The second heavy chain (HC 2) of the construct was comprised of one Fab units (VHCH1) of the anti-OX40 binder 49B4 followed Fc hole chain fused by a (G4S) linker to a VL domain of the anti-FAP binder 28H1 or 4B9. A schematic scheme of the molecules is shown in **Figure 1O**.

b) an antibody with bivalent binding for OX40 as above, wherein the the VH and VL domain of the anti-FAP binder were replaced by a germline control, termed DP47, not binding to the antigen. This molecule is used as negative, “untargeted” control.

c) a bispecific antibody with bivalent binding for OX40 and monovalent binding for FAP was prepared in analogy with example 4.4 of WO 2017/060144 A1 (4+1 format). In this molecule, the first heavy chain (HC 1) was comprised of two Fab units (VHCH1_VHCH1) of the anti-OX40 binder 49B4 followed by Fc knob chain fused by a (G4S) linker to a VH domain of the anti-FAP binder 4B9. The second heavy chain (HC 2) of the construct was comprised of two Fab units (VHCH1_VHCH1) of the anti-OX40 binder 49B4 followed Fc hole chain fused by a (G4S) linker to a VL domain of the anti-FAP binder 4B9. A schematic scheme of the molecule is shown in **Figure 1P**.

Bispecific agonistic Ox40 antibodies with tetravalent binding for Ox40 and monovalent binding for FAP were prepared by applying the knob-into-hole technology to allow the assembling of two different heavy chains. The Pro329Gly, Leu234Ala and Leu235Ala mutations were introduced in the constant region of the heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831 A1.

Table 18: Control molecules used in the experiments

	disclosed in	composed of
OX40 (49B4) FAP (28H1) 2+1 construct (P1AD4356)	Example 4.4 of WO 2017/055398	SEQ ID NO:68, SEQ ID NO:69 2 x SEQ ID NO:70
OX40 (49B4) FAP (4B9) 2+1 construct (P1AD4353, 7719)	Example 4.4 of WO 2017/055398	SEQ ID NO:71, SEQ ID NO:72 2 x SEQ ID NO:70

OX40 (49B4) DP47 2+1 untargeted construct (P1AD4352, 7718)	Example 4.4 of WO 2017/055398	SEQ ID NO:73, SEQ ID NO:74 2 x SEQ ID NO:70
OX40 (49B4) FAP (4B9) 4+1 construct	Example 4.4 of WO 2017/060144 A1	SEQ ID NO:75, SEQ ID NO:76 4 x SEQ ID NO:70

Example 3

Characterization of FAP OX antibodies

3.1 Binding on human OX40 (kinetic affinity)

- 5 Binding of bispecific FAP-OX40 antibodies to human OX40 was investigated by surface plasmon resonance using a BIACORE T100 instrument (GE Healthcare). Around 8000 resonance units (RU) of the capturing system (20 µg/ml anti-human IgG (Fc); Order Code: BR100839; GE Healthcare Bio-Sciences AB, Sweden) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE
- 10 Healthcare. Running buffer was PBS-P pH 7.4 (20 mM phosphate buffer, 2.7 mM KCl, 137 mM NaCl, 0.05% Surfactant P20). The flow cell was set to 25 °C - and the sample block set to 12 °C - and primed with running buffer twice. The bispecific antibody was captured by injecting a 2 µg/ml solution for 60 s at a flow rate of 5 µl/min. Association was measured by injection of human OX40 for 120 s at a flow rate of 30 µl/min starting with 600 nM in 1:3 dilution. The
- 15 dissociation phase was monitored for up to 720 s and triggered by switching from the sample solution to running buffer. The surface was regenerated by washing with 3 M MgCl₂ for 60 s at a flow rate of 10 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from an anti-human IgG (Fc) surface. Blank injections are also subtracted (= double referencing). For calculation of K_D and kinetic parameters the Langmuir 1:1 model was
- 20 used.

Table 19: Binding of anti-FAP/anti-OX40 antibodies to recombinant human OX40

Molecule	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	t 1/2 (min)
Contorsbody CD134-0093	6.82E+05	0.1853	2.72E-07	15.68	0.06
Contorsbody CD134-0094	6.33E+05	0.2051	3.24E-07	15.09	0.06
Control 4+1	5.29E+05	0.1856	3.51E-07	29.41	0.06

Both contorsbodies have similar K_D values compared to a “4+1” IgG-like format for a tetravalent anti-OX40 antibody; affinity is comparable between the molecules and the R_{max} is indicative of the valency of the various molecules tested.

Further FAP-OX40 contorsbodies were tested and showed K_D values as listed in Table 20
5 below.

Table 20: Binding of anti-FAP/anti-OX40 antibodies to recombinant human OX40

Molecule	k_a (1/Ms)	k_d (1/s)	K_D (M)	R_{max} (RU)	$t_{1/2}$ (ses)
Contorsbody 1 (P1AE1122)	1.66E+06	2.12E-01	1.27E-07	11.7	3.3
Contorsbody 2 (P1AE1942)	8.74E+05	2.72E-01	3.11E-07	9.5	2.5
Contorsbody 3 (P1AE1887)	1.16E+06	3.12E-01	2.68E-07	18.9	2.2
Contorsbody 6 (P1AE2340)	1.66E+06	2.90E-01	1.75E-07	9.8	2.4
Contorsbody P1AE0839	2.20E+06	3.62E-01	1.65E-07	6.5	1.9
Contorsbody 4 (P1AE1888)	1.03E+06	2.84E-01	2.76E-07	16.9	2.4
Contorsbody 5 (P1AE2254)	1.83E+06	2.39E-01	1.30E-07	8.7	2.9
Contorsbody 11 (P1AE0821)	8.94E+05	3.30E-01	3.69E-07	9.8	2.1
Contorsbody 8 (P1AE2735)	1.26E+06	2.27E-01	1.80E-07	8.2	3.1
Contorsbody 9 (P1AE2743)	7.30E+05	2.89E-01	3.95E-07	11.5	2.4
Contorsbody 10 (P1AE2762)	1.00E+06	3.30E-01	3.30E-07	9.3	2.1

3.2 Binding on human FAP (kinetic affinity)

Binding of bispecific FAP-OX40 antibodies to human FAP was investigated by surface
10 plasmon resonance using a BIACORE T100 instrument (GE Healthcare). Around 12000
resonance units (RU) of the capturing system (15 μ g/ml anti- histidine antibody; Order Code:
28995056; GE Healthcare Bio-Sciences AB, Sweden) were coupled on a CM5 chip (GE
Healthcare BR-1005-30) at pH 4.5 by using an amine coupling kit supplied by the GE
Healthcare. Running buffer for Immobilization was HBS-N pH 7.4 (10 mM HEPES, 150 mM
15 NaCl, pH 7.4, GE Healthcare). For the following kinetic characterization running buffer was
PBS-P pH 7.4 (20 mM phosphate buffer, 2.7 mM KCl, 137 mM NaCl, 0.05% surfactant P20).

The flow cell was set to 25 °C - and the sample block set to 12 °C - and primed with running buffer twice. The recombinant human FAP was captured by injecting a 25 µg/ml solution for 60 s at a flow rate of 5 µl/min. Association was measured by injection of the bispecific antibody for 120 s at a flow rate of 30 µl/min starting with 300 nM in 1:2 dilution. The dissociation phase was monitored for up to 720 s and triggered by switching from the sample solution to running buffer. The surface was regenerated by washing with 10 mM Glycine pH 1.5 for 60 s at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from an anti-histidine surface. Blank injections are also subtracted (= double referencing). For calculation of K_D and kinetic parameters the Langmuir 1:1 model was used.

10 **Table 21:** Binding of anti-FAP/anti-OX40 antibodies to recombinant human FAP

Molecule	k_a (1/Ms)	k_d (1/s)	K_D (M)	Rmax (RU)	t 1/2 (min)
Contorsbody CD134-0093	3.54E+04	7.08E-04	2.00E-08	21.01	16.32
Contorsbody CD134-0094	1.23E+04	4.62E-04	3.75E-08	28.54	24.98
Control 4+1	4.04E+05	5.84E-04	1.45E-09	28.62	19.80

Both molecules have similar K_D values. The association to the FAP ECD was less optimal in the Contorsbody format compared to a standard control molecule. However, in the control molecule, two 4xG4S peptide linker were used to link the anti-FAP moiety C-terminally to the Fc part, whereas in the contorsbodies two 2xG4S peptide linker were used to link the anti-FAP moiety to the contorsbody. Further FAP-OX40 contorsbodies were tested and showed K_D values as listed in Table 22 below.

15 **Table 22:** Binding of anti-FAP/anti-OX40 antibodies to recombinant human FAP

Molecule	k_a (1/Ms)	k_d (1/s)	K_D (M)	Rmax (RU)	t 1/2 (sec)
Contorsbody 4 (P1AE1888)	5.49E+04	1.95E-04	3.55E-09	29.9	3557.8
Contorsbody P1AE0839	4.74E+04	1.83E-04	3.87E-09	17.2	3778.1
Contorsbody 1 (P1AE1122)	5.29E+04	2.02E-04	3.81E-09	26.1	3432.9
Contorsbody 2 (P1AE1942)	4.71E+04	2.54E-04	5.39E-09	18.5	2727.1
Contorsbody 5 (P1AE2254)	4.79E+04	2.66E-04	5.54E-09	20.6	2610.2
Contorsbody 6 (P1AE2340)	1.96E+05	2.18E-04	1.11E-09	28.1	3176
Contorsbody 3 (P1AE1887)	5.34E+04	2.06E-04	3.86E-09	35.4	3362.1
Contorsbody 11	5.18E+04	3.03E-04	5.86E-09	16.9	2285

(P1AE0821)					
Contorsbody 8 (P1AE2735)	2.91E+05	5.13E-04	1.76E-09	33.4	1352.2
Contorsbody 9 (P1AE2743)	3.01E+05	4.86E-04	1.62E-09	36.9	1425.6
Contorsbody 10 (P1AE2762)	3.01E+05	3.59E-04	1.19E-09	40.9	1932.4

3.3 Simultaneous Binding on human OX40 and human FAP (kinetic affinity)

The capacity of binding simultaneously human OX40 and human FAP was also assessed by surface plasmon resonance (SPR) using a BIACORE T100 instrument (GE Healthcare).

- 5 Around 8000 resonance units (RU) of the capturing system (20 µg/ml anti-human IgG (Fc); Order Code: BR100839; GE Healthcare Bio-Sciences AB, Sweden) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. Running buffer was PBS-P pH 7.4 (20 mM phosphate buffer, 2.7 mM KCl, 137 mM NaCl, 0.05% Surfactant P20). The flow cell was set to 25 °C - and the sample block set to 12 °C
- 10 - and primed with running buffer twice. The bispecific antibody was captured by injecting a 2 µg/ml solution for 60 seconds at a flow rate of 5 µl/min. Association was measured by injection of the first analyte (human OX40 or human FAP, respectively) for 120 seconds at a flow rate of 30 µl/min. Then the second analyte (human FAP or human OX40, respectively) was injected with a flow rate of 30 µl/min for 120 seconds. The dissociation phase was monitored for up to
- 15 720 seconds and triggered by switching from the sample solution to running buffer. The surface was regenerated by washing with 3 M MgCl₂ for 60 seconds at a flow rate of 10 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from an anti-human IgG (Fc) surface. Blank injections are also subtracted (= double referencing). For calculation of K_D and kinetic parameters the Langmuir 1:1 model was used. All FAP-OX40
- 20 contorsbodies were able to bind simultaneously and independently to both antigens.

Example 4

Binding on cells

4.1 Binding to naïve versus activated human PBMCs

- 25 Buffy coats were obtained from the Zürich blood donation center. Human PBMC were isolated by ficoll density gradient centrifugation. To isolate fresh peripheral blood mononuclear cells (PBMCs) the buffy coat was diluted with the same volume of DPBS (Gibco by Life Technologies, Cat. No. 14190 326). 50 mL polypropylene centrifuge tubes (TPP, Cat. No. 91050) were supplied with 15 mL Histopaque 1077 (SIGMA Life Science, Cat. No. 10771, polysucrose

and sodium diatrizoate, adjusted to a density of 1.077 g/mL) and the buffy coat solution was layered above the Histopaque 1077. The tubes were centrifuged for 30 min at 400 x g, room temperature and with low acceleration and no break. Afterwards the PBMCs were collected from the interface, washed three times with DPBS and resuspended in T cell medium consisting of

5 RPMI 1640 medium (Gibco by Life Technology, Cat. No. 42401-042) supplied with 10 % Fetal Bovine Serum (FBS, Gibco by Life Technology, Cat. No.16000-044, Lot 941273, gamma-irradiated, mycoplasma-free and heat inactivated at 56 °C for 35 min), 1 % (v/v) GlutaMAX I (GIBCO by Life Technologies, Cat. No. 35050 038), 1 mM Sodium Pyruvate (SIGMA, Cat. No. S8636), 1 % (v/v) MEM non-essential amino acids (SIGMA, Cat.-No. M7145) and 50 µM β-

10 Mercaptoethanol (SIGMA, M3148). PBMCs were frozen in FBS containing 10% (v/v) dimethyl sulfoxide.

Frozen PBMCs were thawed in T cell medium and PBMCs were used directly after isolation (binding on resting human PBMCs) or they were stimulated to receive a strong human OX40 expression on the cell surface of T cells (binding on activated human PBMCs). Therefore

15 naïve PBMCs were cultured for two days in T cell medium supplied with 200 U/mL Proleukin and 2 µg/mL PHA-L in 6-well tissue culture plate and then 1 day on pre-coated 6-well tissue culture plates [4 µg/mL anti-human CD3 (clone OKT3) and 2 µg/mL anti-human CD28 (clone CD28.2)] in T cell medium.

For detection of OX40 naïve human PBMC and activated human PBMC were mixed. To

20 enable distinction of naïve from activated human PBMC naïve cells were labeled prior to the binding assay using the eFluor670 cell proliferation dye (eBioscience, Cat.-No.65-0840-85). A 1 to 1 mixture of 1×10^5 naïve, eFluor670 labeled human PBMC and unlabeled activated human PBMC were then added to each well of a round-bottom suspension cell 96-well plates (greiner bio-one, cellstar, Cat. No. 650185) and the binding assay was performed.

25 Cells were stained for 120 minutes at 4 °C in the dark in 50 µL/well 4 °C cold FACS buffer containing titrated anti-Ox40 antibody constructs. After three times of washing with excess FACS buffer, cells were stained for 45 minutes at 4 °C in the dark in 25 µL/well 4 °C cold FACS buffer containing a mixture of fluorescently labeled anti-human CD4 (clone RPA-T4, mouse IgG1 k, BioLegend, Cat.-No. 300532), anti-human CD8 (clone RPA-T8, mouse IgG1k,

30 BioLegend, Cat.-No. 3010441) and Fluorescein isothiocyanate (FITC)-conjugated AffiniPure anti-human IgG Fcγ-fragment-specific goat IgG F(ab')₂ fragment (Jackson ImmunoResearch, Cat.-No. 109-096-098). Plates were finally resuspended in 85 µL/well FACS-buffer containing 0.2 µg/mL DAPI (Santa Cruz Biotec, Cat. No. Sc-3598) and acquired the same day using 5-laser LSR-Fortessa (BD Bioscience with DIVA software).

35 As shown in **Figures 2B and 2D**, no antigen binding molecule specific for OX40 bound to resting human CD4 T-cells or CD8 T-cells. In contrast, all antigen binding molecules (OX40 (49B4) FAP (28H1) 2+1 bispecific antibody, OX40 (49B4) DP47 2+1 bispecific antibody, OX40 (49B4) FAP (4B9) 4+1 bispecific antibody, Contorsbodies CD134-0093 and CD134-0094)

bound to activated CD8⁺ or CD4⁺ T-cells (**Figures 2A and 2C**). Binding to CD4⁺ T-cells was much stronger than that to CD8⁺ T cells. All formats of a 2+1 design bound with similar strength to OX40 expressing (positive) cells, independently of the binding moiety of the second specificity. Additionally, the 4+1 construct showed the strongest binding. The Contorsbody CD134-0093 showed an intermediate binding between the 2+1 and 4+1 formats, the second Contorsbody CD134-0094 bound less strong to CD4⁺ and CD8⁺ T-cells than the 2+1 formats. The negative control DP47 hu IgG1 antibody (P329G LALA) did not bind to activated nor to resting T-cells. Since OX40 is not upregulated on resting CD4 or CD8 T-cells, none of the tested molecules bound to resting cells. Moreover, binding of all constructs (except the negative control) was stronger on CD4 T-cells, since OX40 expression is higher on these cells than on CD8 T-cells.

The results of a second experiment are shown in **Figures 2E to 2H**. As shown in these figures, none of the molecules tested (OX40 (49B4) FAP (28H1) 2+1 bispecific antibody, OX40 (49B4) FAP (4B9) 2+1 bispecific antibody, OX40 (49B4) DP47 2+1 bispecific antibody, Contorsbodies P1AE0085, P1AE0086 and P1AE0087) bound to resting human CD4⁺ T-cells or CD8⁺ T-cells as expected since OX40 is not expressed on resting cells (**Figures 2F and 2H**). In contrast, all antigen binding molecules displayed binding to activated CD8⁺ and CD4⁺ T-cells (**Figures 2E and 2G**). The signal amplitude was lower on CD8⁺ T cells than on CD4 T cells which correlated with OX40 expression level (high on CD4⁺ T cells). However, binding pattern of each tested molecule was comparable between CD4⁺ and CD8⁺ T cells. All three contorsbody molecules did bind to OX40 expressing cells, however they showed reduced binding capacity to OX40 as compared to the 2+1 control molecules, irrespective of the FAP binding clone. In particular, the binding of contorsbody P1AE0087 was slightly more impaired than that of contorsbody P1AE0085.

The binding of further contorsbodies (Contorsbodies 1 to 11) to activated CD4⁺ T-cells is shown in **Figures 8A to 8D** and binding to activated CD8⁺ T-cells is shown in **Figures 10A to 10D**. In **Figures 9A to 9D** and **Figures 11A to 11D**, respectively, it is shown that none of Contorsbodies 1 to 11 bound to resting human CD4 T-cells or CD8 T-cells as expected. In contrast, all antigen binding molecules bound to activated CD8⁺ or CD4⁺ T-cells. Binding to CD4⁺ T-cells was much stronger than that to CD8⁺ T cells. All formats of a 2+1 design bound with similar strength to OX40 positive cells, independently of the binding moiety of the second specificity. The negative control (DP47 hu IgG1 P329G LALA) did not bind to activated nor resting T-cells. Since OX40 is not upregulated on resting CD4 or CD8 T-cells, none of the tested molecules bound to resting cells. Moreover, binding of all constructs (except the negative control) was stronger on CD4⁺ T-cells, since OX40 expression is higher on these cells than on CD8⁺ T-cells.

4.2 Binding to human FAP-expressing tumor cells

The binding to cell surface FAP was tested using human fibroblast activating protein (huFAP) expressing WM266-4 cells (ATCC CRL-1676). The lack of binding to OX40 negative FAP negative tumor cells was tested using A549 NucLight™ Red Cells (Essen Bioscience, Cat. No. 4491) expressing the NucLight Red fluorescent protein restricted to the nucleus to allow separation from unlabeled human FAP positive WM266-4 cells. Parental A549 (ATCC CCL-185) were transduced with the Essen CellPlayer NucLight Red Lentivirus (Essen Bioscience, Cat. No. 4476; EF1 α , puromycin) at an MOI of 3 (TU/cell) in the presence of 8 μ g/ml polybrene following the standard Essen protocol. This resulted in $\geq 70\%$ transduction efficiency.

A mixture of 5×10^4 unlabeled WM266-4 cells and unlabeled A549 NucLight™ Red Cells in FACS buffer were added to each well of a round-bottom suspension cell 96-well plates (greiner bio-one, cellstar, Cat. No. 650185) and the binding assay was performed. Plates were centrifuged 4 minutes, 400 x g at 4 °C and supernatants were flicked off. Cells were washed once with 200 μ L DPBS and pellets were resuspended by a short and gentle vortex. All samples were resuspended in 50 μ L/well of 4 °C cold FACS buffer containing the bispecific antigen binding molecules (primary antibody) at the indicated range of concentrations (titrated) and incubated for 120 minutes at 4 °C. Afterwards the cells were washed four times with 200 μ L 4 °C FACS buffer and resuspended by a short vortex. Cells were further stained with 25 μ L/well of 4 °C cold secondary antibody solution containing Fluorescein isothiocyanate (FITC)-conjugated AffiniPure anti-human IgG Fc γ -fragment-specific goat IgG F(ab')₂ fragment (Jackson ImmunoResearch, Cat. No. 109-096-098) and incubated for 60 minutes at 4 °C in the dark. Plates were finally resuspended in 90 μ L/well FACS-buffer containing 0.2 μ g/mL DAPI (Santa Cruz Biotech, Cat. No. Sc-3598) and acquired the same day using 5-laser LSR-Fortessa (BD Bioscience with DIVA software).

As shown in **Figures 3A and 3B**, the FAP-targeted anti-OX40 bispecific antibodies bound efficiently to human FAP-expressing target cells. Therefore, only FAP- targeted anti-OX40 antigen binding molecules show direct tumor-targeting properties. The FAP 4B9 has a high affinity to human FAP, whereas the 28H1 has a low affinity to human FAP. The FAP-targeted (OX40 (49B4) FAP (4B9) 4+1 bispecific antibody indicated the strongest binding to FAP⁺ cells, followed by the 2+1 Contorsbody CD134-0093, Contorsbody CD134-0094 and then the OX40 (49B4) FAP (28H1) 2+1 bispecific antibody. It has to be noted that FAP 4B9 has a higher affinity to human FAP compared to 28H1. The binding to FAP⁺ cells of both contorsbodies was slightly improved compared to a VHVL containing targeted 2+1 antibody construct. The non-targeted OX40 (49B4) DP47 2+1 bispecific antibody and the negative control (DP47 hu IgG1 antibody (P329G LALA)) did not bind to any FAP⁺ cells. EC₅₀ values of binding to activated human CD4 T cells and FAP positive tumor cells are summarized in Table 23.

Table 23: EC₅₀ values for binding of FAP targeted OX40 (49B4) bispecific antibodies in different formats to cell surface human FAP and human OX40 (on CD4⁺ T-cells)

Format	FAP ⁺ cell EC ₅₀ [nM]	OX40 ⁺ cell EC ₅₀ [nM]
OX40 (49B4) FAP (4B9) 4+1 construct	11.342	0.127
Contorsbody 1 (CD134-0093)	61.231	1.693
Contorsbody 2 (CD134-0094)	227.281	4.988
OX40 (49B4) FAP (28H1) 2+1 construct	78.873	7.023
OX40 (49B4) DP47 2+1 construct control	-	-

In a further experiment, Contorsbodies P1AE0085, P1AE0086 and P1AE0087 were tested in comparison with OX40 (49B4) FAP (28H1) 2+1 bispecific antibody, OX40 (49B4) FAP (4B9) 2+1 bispecific antibody and OX40 (49B4) DP47 2+1 bispecific antibody (negative control). The results are shown in **Figures 3C and 3D**. All FAP-targeted anti-OX40 antigen binding molecules bound to human FAP-expressing cells. The FAP 4B9 clone has a higher affinity to human FAP as the 28H1. Consequently, the best binding properties were observed with OX40 (49B4) FAP (4B9) 2+1 bispecific antibody and OX40 (49B4) FAP (28H1) 2+1 bispecific antibody showed reduced binding as expected. All three contorsbodies showed binding to FAP, although the binding to FAP⁺ cells was slightly impaired compared to the OX40 (49B4) FAP 2+1 bispecific antibodies for both FAP clones (4B9 and 28H1). The linker length did not seem to affect the binding to huFAP since contorsbody P1AE0085 and P1AE0087 displayed very similar binding properties. The non-targeted 2 OX40 (49B4) DP47 2+1 bispecific antibody and the negative control (DP47 hu IgG1 antibody (P329G LALA)) did not bind to any FAP⁺ cells. In addition, none of the molecules showed binding to FAP- A549NLR cells, indicating that the binding was specific to human FAP. EC₅₀ values of binding to activated human CD4 and CD8 T cells and FAP positive cells are summarized in Table 24.

Table 24: EC₅₀ values for binding of FAP targeted OX40 (49B4) bispecific antibodies in different formats to cell surface human FAP and human OX40 (on CD4⁺ T-cells)

Format	FAP ⁺ cell EC ₅₀ [nM]	OX40 ⁺ CD4 ⁺ T cell EC ₅₀ [nM]	OX40 ⁺ CD8 ⁺ T cell EC ₅₀ [nM]
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Contorsbody P1AE0085 (28H1)	102.34	n.c	n.c
Contorsbody P1AE0086 (4B9)	5.48	n.c	n.c
Contorsbody P1AE0087 (28H1)	42.26	63.89	7.30
OX40 (49B4) FAP (28H1) 2+1 bispecific antibody	14.94	0.20	0.30
OX40 (49B4) FAP (4B9) 2+1 bispecific antibody	0.75	0.22	0.31
OX40 (49B4) DP47 2+1 bispecific antibody	n.a	0.47	1.79

n.c. No curve fit. EC₅₀ calculation not possible

n.a. Not applicable

The binding results for Contorsbodies 1 to 10 to FAP⁺ cells (NIH/3T3-huFAP tumor cells) are shown in **Figures 13A to 13D**. All FAP-targeted anti-OX40 antigen binding molecules bound to human FAP-expressing cells. As shown in **Figures 14A to 14D**, none of the FAP-targeted anti-OX40 antigen binding molecules were able to bind to A549NLR (FAP negative) tumor cells. Contorsbody 8 bound strongest to FAP⁺ cells, followed by Contorsbody 10 and Contorsbody 6. The binding to FAP⁺ cells of both contorsbodies was slightly improved compared to a VHVL containing targeted 2+1 antibody construct. The non-targeted 2+1 anti OX40 construct (7718) and the negative control (8105) did not bind to any FAP⁺ cells. EC₅₀ values of binding to activated human CD4⁺ T cells and FAP positive tumor cells are summarized in Table 25.

Table 25: EC₅₀ values for binding of FAP targeted OX40 (49B4) bispecific antibodies in different formats to cell surface human FAP and human Ox40 (on CD4⁺ T-cells)

Format	FAP ⁺ cell EC ₅₀ [nM]	OX40 ⁺ CD4 ⁺ T cell EC ₅₀ [nM]
Contorsbody 1 (P1AE1122)	4.45	0.257
Contorsbody 2 (P1AE1942)	6.86	1.02
Contorsbody 3 (P1AE1887)	7.68	25.39
Contorsbody 4 (P1AE1888)	6.74	31.82
Contorsbody 5 (P1AE2254)	11.74	0.677
Contorsbody 6 (P1AE2340)	0.74	n.c.

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Contorsbody 7 (P1AE0086)	2.47	0.126
Contorsbody 8 (P1AE2735)	0.736	7.02
Contorsbody 9 (P1AE2743)	0.398	2.56
Contorsbody 10 (P1AE2762)	0.464	1.22
OX40 (49B4) DP47 2+1 bispecific antibody (P1AD4352)	n.d.	0.086
OX40 (49B4) FAP (4B9) 2+1 bispecific antibody (P1AD4353)	4.46	n.d.
OX40 (49B4) DP47 4+1 bispecific antibody (P1AD4524)	3.98	0.0001

n.c. No curve fit. EC₅₀ calculation not possible

n.a. Not applicable

Example 5

Functional properties of bispecific anti-human OX40 binding molecules

5.1 HeLa cells expressing human OX40 and reporter gene NFκB-luciferase

Agonistic binding of OX40 to its ligand induces downstream signaling via activation of nuclear factor kappa B (NFκB) (A. D. Weinberg et al., J. Leukoc. Biol. 2004, 75(6), 962-972). The recombinant reporter cell line HeLa_hOx40_NFκB_Luc1 was generated to express human OX40 on its surface. Additionally, it harbors a reporter plasmid containing the luciferase gene under the control of an NFκB-sensitive enhancer segment. OX40 triggering induces dose-dependent activation of NFκB, which translocates in the nucleus, where it binds on the NFκB sensitive enhancer of the reporter plasmid to increase expression of the luciferase protein. Luciferase catalyzes luciferin-oxidation resulting in oxyluciferin which emits light. This can be quantified by a luminometer.

Thus, the capacity of the various anti-OX40 molecules to induce NFκB activation in HeLa_hOx40_NFκB_Luc1 reporter cells was analyzed as a measure for bioactivity.

The NFκB activating capacity of selected bispecific OX40(49B4) antibodies (in a bivalent FAP-targeted V_HV_L or Contorsbody format alone and with hyper-crosslinking of the constructs by either a secondary antibody or a FAP+ tumor cell line was tested. The crosslinking of FAP-binding antibodies by cell surface FAP was tested using human fibroblast activating protein (huFAP) expressing NIH/3T3-huFAP clone 19. This cell line was generated by the transfection of the mouse embryonic fibroblast NIH/3T3 cell line (ATCC CRL-1658) with the expression vector pETR4921 to express huFAP under 1.5μg/mL Puromycin selection.

Adherent HeLa_hOX40_NFκB_Luc1 cells were cultured over night at a cell density of 0.2×10^5 cells per well and were stimulated for 5 hours with assay medium containing titrated bispecific anti-OX40 (49B4) antibodies (OX40 (49B4) FAP (28H1) 2+1 bispecific antibody, OX40 (49B4) DP47 2+1 bispecific antibody, Contorsbodies CD134-0093 and CD134-0094). For testing the effect of hyper-crosslinking by secondary antibodies, 25 μL/well of medium containing secondary antibody anti-human IgG Fcγ-fragment-specific goat IgG F(ab')₂ fragment (Jackson ImmunoResearch, 109-006-098) was added in a 1:2 ratio (primary to secondary antibodies). To test the effect of hyper-crosslinking by cell surface FAP binding, 25 μL/well of medium containing FAP+ tumor cells (NIH/3T3-huFAP clone 19) were co-cultured in a 4 to 1 ratio (four time as much FAP+ tumor cells than reporter cells per well).

After incubation, assay supernatant was aspirated and plates washed two times with DPBS. Quantification of light emission was done using the luciferase 100 assay system and the reporter lysis buffer (both Promega, Cat.-No. E4550 and Cat-No: E3971) according to manufacturer instructions. Briefly, cells were lysed for 10 minutes at -20 °C by addition of 30 μL per well 1x lysis buffer. Cells were thawed for 20 minutes at 37 °C before 90 μL per well provided luciferase assay reagent was added. Light emission was quantified immediately with a SpectraMax M5/M5e microplate reader (Molecular Devices, USA) using 500ms integration time, without any filter to collect all wavelengths. Emitted relative light units (URL) were corrected by basal luminescence of HeLa_hOX40_NFκB_Luc1 cells and were blotted against the logarithmic primary antibody concentration using Prism4 (GraphPad Software, USA). Curves were fitted using the inbuilt sigmoidal dose response.

As shown in **Figures 4A to 4C**, the presence of all anti-OX40 constructs induced NFκB activation. Hyper-crosslinking via secondary anti-huIgG Fcγ-specific antibody increased NFκB activation for all binders in a FAP independent manner. The two bispecific antibodies, 2+1 constructs OX40 (49B4) FAP (28H1) and OX40 (49B4) DP47, ran similar because the FAP targeting seemed not to impact the NFκB induction. The Contorsbodies CD134-0093 and CD134-0094 performed comparable, but showed a less strong NFκB activation under crosslinking (their effect seems to be intrinsic and not crosslinking dependant). FAP-expressing tumor cells strongly increased induction of NFκB-mediated luciferase-activation in a concentration-dependent manner when FAP targeted molecules (filled triangle, semi-filled circle, filled circle) were used. No such effect was seen with the OX40 (49B4) DP47 2+1 bispecific antibody (open circle) as the construct could not be further hyper-crosslinked by FAP+ tumor cells. Additionally, the FAP targeted OX40 (49B4) FAP (28H1) 2+1 bispecific antibody induced a stronger NFκB activation than Contorsbody CD134-0093 and Contorsbody CD134-0094, whereas the CD134-0094 showed stronger activation than the CD134-0093 (Figure 4A). Altogether, the contorsbody constructs were able to induce OX40 mediated NFκB activation however they were less active than FAP targeted OX40 (49B4) FAP (28H1) 2+1 bispecific

antibody, either crosslinked by secondary antibody or cell surface expressed FAP. However, there was also less activity when added w/o secondary oligomerization.

The results of a second experiment are shown in **Figures 4D to 4F**. As shown in these figures, the presence of all anti-OX40 molecules tested (OX40 (49B4) FAP (28H1) 2+1 bispecific antibody, OX40 (49B4) FAP (4B9) 2+1 bispecific antibody, OX40 (49B4) DP47 2+1 bispecific antibody, Contorsbodies P1AE0085, P1AE0086 and P1AE0087) induced a very minimal NFκB activation in the absence of cross-linking (Figure 4D). All three 2+1 control molecules (OX40 (49B4) FAP (28H1) 2+1 bispecific antibody, OX40 (49B4) FAP (4B9) 2+1 bispecific antibody and OX40 (49B4) DP47 2+1 bispecific antibody) showed very similar NFκB activation when hyper-crosslinking was provided by a secondary antibody (Figure 4F). The three contorsbody molecules showed a slightly reduced activity as compared to the controls. This difference in NFκB activation between the tested contorsbody and control molecules was not observed in the presence of FAP-expressing cells. The curves corresponding to the contorsbody molecules and their respective FAP-targeted control molecules were analyzed with GraphPad Prism software as sharing one global curve fit, indicating that there was no significant difference in potency. Even though there is a different binding affinity for FAP between the two clones tested (4B9 and 28H1), it did not translate into a noticeable difference in potency in this assay. DP47-targeted 2+1 control molecule displayed only minimal activity since it does not have an FAP binding moiety. The negative control (DP47 hu IgG1 antibody (P329G LALA)) showed no activity, independent of cross-linking.

We further tested the NFκB activating capacity of Contorsbodies 1 to 11 alone and with hyper-crosslinking of the bispecific antibody constructs by the FAP⁺ tumor cell line. In **Figures 15A to 15D** is shown the NFκB activation in the presence of FAP expressing tumor cells. The NFκB activation without crosslinking by FAP can be seen in **Figures 16A to 16D**. A summary of the area under the curve values for NFκB activation in HeLa cells with and without crosslinking with FAP⁺ cells is shown in **Figure 17**. The presence of all anti-OX40 constructs induced NFκB activation. Hyper-crosslinking via FAP⁺ tumor cells increased NFκB activation for all tested bispecific antibodies. FAP-expressing tumor cell strongly increased induction of NFκB-mediated luciferase-activation in a concentration-dependent manner when FAP targeted molecules were used. No such effect was seen when in the 2+1 format the FAP binding moiety was replaced by a non-binding DP47 unit (open triangle) as the construct could not be further hyper-crosslinked by FAP⁺ tumor cells. All of the contorsbodies performed comparably to the OX40 (49B4) FAP (4B9) 2+1 bispecific antibody.

5.2 Ox40 mediated co-stimulation of sub-optimally TCR triggered resting human PBMC and hyper-crosslinking by cell surface FAP

It was shown in section 5.1 that addition of FAP⁺ tumor cells can strongly increase the NFκB activity induced by FAP targeted bivalent anti-OX40 antibodies in human OX40 positive reporter cell lines by providing strong oligomerization of OX40 receptors. Likewise, we tested FAP-targeted bivalent anti-OX40 antibodies in the presence of NIH/3T3-huFAP clone 19 cells for their ability to rescue suboptimal TCR stimulation of resting human PBMC cells.

Human PBMC preparations contain (1) resting OX40 negative CD4⁺ and CD8⁺ T cells and (2) antigen presenting cells with various Fcγ receptor molecules on their cell surface e.g. B cells and monocytes. Anti-human CD3 antibody of human IgG1 isotype can bind with its Fc part to the present Fcγ receptor molecules and mediate a prolonged TCR activation on resting OX40 negative CD4⁺ and CD8⁺ T cells. These cells then start to express OX40 within several hours. Functional agonistic compounds against OX40 can signal via the OX40 receptor present on activated CD8⁺ and CD4⁺ T cells and support TCR-mediated stimulation.

Resting human PBMC were stimulated for five days with a suboptimal concentration of anti-CD3 antibody in the presence of irradiated FAP⁺ NIH/3T3-huFAP clone 19 cells and titrated anti-OX40 constructs. Effects on T-cell survival and proliferation were analyzed through monitoring of total cell counts and co-staining with fluorescently-labeled antibodies against T-cell activation and maturation markers (CD25 / CD127) by flow cytometry.

Mouse embryonic fibroblast NIH/3T3-huFAP clone 19 cells were harvested using cell dissociation buffer (Invitrogen, Cat.-No. 13151-014) for 10 minutes at 37 °C. Cells were washed once with DPBS. NIH/3T3-huFAP clone 19 cells were cultured at a density of 0.2*10⁵ cells per well in T cell media in a sterile 96-well round bottom adhesion tissue culture plate (TPP, Cat. No 92097) over night at 37 °C and 5% CO₂ in an incubator (Hera Cell 150). The next day they were irradiated in an xRay irradiator using a dose of 4500 RAD to prevent later overgrowth of human PBMC by the tumor cell line.

Human PBMCs were isolated by ficoll density centrifugation. Cells were added to each well at a density of 0.6*10⁵ cells per well. Anti-human CD3 antibody (clone V9, human IgG1) at a final concentration of [10 nM] and FAP targeted bivalent anti-OX40 antigen binding molecules and Contorsbodies were added at the indicated concentrations. Cells were activated for four days at 37 °C and 5% CO₂ in an incubator (Hera Cell 150).

Then, cells were surface-stained with fluorescent dye-conjugated antibodies anti-human CD4 (clone RPA-T4, BioLegend, Cat.-No. 300532), CD8 (clone RPa-T8, BioLegend, Cat.-No. 3010441), CD25 (clone M-A251, BioLegend, Cat.-No. 356112) and CD127 (clone A019D5, BioLegend, Cat.-No. 351324) for 20 min at 4°C. Cell pellets were washed once with FACS buffer. Plates were finally resuspended in 85 µL/well FACS-buffer containing 0.2 µg/mL DAPI

(Santa Cruz Biotech, Cat. No. Sc-3598) and acquired the same day using 5-laser LSR-Fortessa (BD Bioscience with DIVA software).

As shown in **Figures 5A to 5F**, co-stimulation with non-targeted OX40 (49B4) DP47 2+1 bispecific antibody (open triangle) did not rescue sub-optimally TCR stimulated CD4 and CD8 T cells. Hyper-crosslinking of the FAP targeted OX40 (49B4) FAP (28H1) 2+1 bispecific antibody (filled triangle) and the 2+1 Contorsbodies CD134-0093 and CD134-0094 by the presence of NIH/3T3-huFAP clone 19 cells strongly promoted survival and induced an enhanced activated phenotype in human CD4 and CD8 T cells. Furthermore, especially the Contorsbody CD134-0093 (filled circle) seems to show the most similar properties compared to the targeted OX40 (49B4) FAP (28H1) 2+1 bispecific antibody regarding activation. Moreover, the Contorsbody CD134-0094 (half filled circles) showed a stronger activation for CD8 T-cells than for CD4 T-cells (Figure 5D), whereas it demonstrated comparable activation to the Contorsbody CD134-0093 and the FAP targeted OX40 (49B4) FAP (28H1) 2+1 bispecific antibody on CD4 T-cells. Altogether, results of the bioactivity and T-cell activation were normalized and summarized in **Figure 6**, where the agonistic capacity of each construct was quantified for the analyzed markers as area under the curve and plotted against each other. As shown in Figure 6, the Contorsbody CD134-0093 showed the strongest activation of both CD4 and CD8 T-cells regarding FSC-A and CD25. This molecule seems to be more potent than the FAP targeted OX40 (49B4) FAP (28H1) 2+1 bispecific antibody, which is roughly about 75% as strong as CD134-0093. The Contorsbody CD134-0094 seemed to be a bit less potent, since only CD25 got upregulated, but the size (FSC-A) of CD4 and CD8 T cells did not increase. Furthermore, the untargeted 2+1 molecule and the negative control did not show any activation (very low normalized AUC values).

In a further experiment, co-stimulation with non-targeted OX40 (49B4) DP47 2+1 bispecific antibody did not rescue sub-optimally TCR stimulated CD4⁺ and CD8⁺ T-cells. Hyper-crosslinking of the FAP targeted bivalent anti-OX40 antibodies OX40 (49B4) FAP (28H1) 2+1 bispecific antibody and OX40 (49B4) FAP (4B9) bispecific antibody, and the three contorsbody molecules (P1AE0085, P1AE0086 and P1AE 0087) by the presence of NIH/3T3-huFAP clone 19 cells strongly promoted an enhanced activation of primary human CD4 and CD8 T cells. P1AE0086 triggered a very similar activation of T cells than the corresponding 2+1 4B9 control antibody OX40 (49B4) FAP (4B9) bispecific antibody. They led to a more activated phenotype than the molecules with 28H1 FAP binder that is known to have a lower affinity for human FAP. Contorsbodies P1AE0085 and P1AE0087 showed a similar CD25 expression on CD4 and CD8 T-cells and a slightly higher FSC-A MFI as compared to 2+1 control molecule OX40 (49B4) FAP (28H1) 2+1 bispecific antibody. This assay with primary PBMCs showed that the potency of FAP-targeted molecules containing the high affinity binder (4B9) is higher

than that of the molecules with low affinity FAP binder (28H1). The results of the bioactivity and T-cell activation are shown in **Figures 7A to 7D**.

The ability of Contorsbodies 7, 8, 9 and 10 to rescue sub-optimally TCR stimulated CD4⁺ and CD8⁺ T-cells was also tested. **Figures 18A and 18B** show the activation of CD4 T cells indicated by the surface marker CD25 after suboptimal CD3 stimulation. In **Figures 18C and 18D** the activation of CD8 T cells indicated by the surface marker CD25 is shown. **Figures 19A and 19B** show the FSC-A MFI of CD4 T cells and **Figures 19C and 19D** the the FSC-A MFI of CD8 T cells. eFluor 670 is a proliferation dye for the measurement of cell divisions. The eFluor 670 levels, respectively the proliferation of CD4 T cells is shown in **Figures 20A and 20B** and the proliferation of CD8 T cells is shown in **Figures 20C and 20D**. The downregulation of IL-7Ra (CD127) is shown for CD4 T cells in **Figures 21A and 21B** and for CD8 T cells in **Figures 21C and 21D**. All results demonstrate that hyper-crosslinking of the contorsbodies and OX40 (49B4) FAP (4B9) bispecific antibody (filled triangle) in the presence of NIH/3T3-huFAP clone 19 cells strongly promoted survival and induced an enhanced activated phenotype in human CD4 and in a smaller degree in CD8 T cells. In **Figures 22A to 22D** the results of the various bioactivity assays are summarized as area under the curve.

5.3 Summary of results

Altogether, the 2+1 Contorsbody CD134-0093 performed better than the 2+1 Contorsbody CD134-0094. The binding of CD134-0093 on activated CD4 T-cells was stronger than the one of the FAP targeted OX40 (49B4) FAP (28H1) 2+1 bispecific antibody and of CD134-0094, which both showed similar binding properties (Fig. 2A). Both Contorsbodies demonstrated good binding to human surface FAP on WM266-4 cells (Fig. 3A), the binding strength was between the ones from OX40 (49B4) FAP (4B9) 4+1 bispecific antibody (comprising the high affinity binder 4B9) and OX40 (49B4) FAP (28H1) 2+1 bispecific antibody (comprising the low affinity binder 28H1). Even if the format of the contorsbodies is different from the OX40 (49B4) FAP (28H1) 2+1 bispecific antibody as describe din Example 2.9, the binding properties seem to be as good or even better. Nevertheless, they do not reach the MFIs of the OX40 (49B4) FAP (4B9) 4+1 bispecific antibody (Example 2.9). Looking at NFκB activation with crosslinking NIH 3T3 huFAP cells, the Contorsbodies showed an intermediate activation of NFκB compared to the (49B4) FAP (28H1) 2+1 bispecific antibody, but still stronger than the untargeted OX40 (49B4) DP47 2+1 bispecific antibody (Fig 4A). The FAP domain on both Contorsbodies seems to be able to act as crosslinkers and activate OX40 signaling. Using a secondary Fcγ specific antibody as crosslinker, both Contorsbodies demonstrated similar NFκB activation, but performed less good than the targeted control molecules (Fig. 4B). The bioactivity testing of the Contorsbodies revealed that CD134-0093 seemed to induce a more pronounced activated phenotype on CD4

and CD8 T-cells than CD134-0094 (Fig. 5A-D). The Contorsbody CD134-0093 even demonstrated a stronger activation of T-cells than the bivalent 2+1 format (Fig. 6).

Contorsbodies P1AE0085, P1AE0086 and P1AE 0087 showed a slightly reduced capacity to bind to OX40 expressed on the surface of activated CD4⁺ and CD8⁺ T-cells, as compared to bivalent anti OX40 control molecules (Figures 2E-2H). P1AE0085, P1AE0086 and P1AE0087 also displayed a partially impaired binding to human FAP expressed on the surface of NIH-3T3 huFAP clone 19 cells (Figures 3C and 3D) in comparison to their respective controls (2+1 4B9 and 28H1 FAP binders). The molecules containing the high affinity FAP binder 4B9 demonstrated a superior binding capacity to human FAP than that of the molecules with the lower affinity 28H1 binder. The NFκB activation assay revealed that the three contorsbody molecules P1AE0085, P1AE0086 and P1AE 0087 induced a similar activation of NFκB than the control FAP-targeted molecules when cross-linked via NIH-3T3 huFAP clone 19 cells (Figure 4F). There was no discrimination in the potency of the low and high affinity FAP-targeted anti-OX40 molecules in the HeLa NFκB reporter assay. The bioactivity of the contorsbody molecules was also tested using primary human PBMCs and NIH-3T3 huFAP clone 19 cells as cross-linking cells in a five-day activation assay. Molecules with 4B9 FAP binder induced a more activated phenotype in CD4⁺ and CD8⁺ T-cells as compared to 28H1 binder. The contorsbody molecules performed as well as their respective control molecules. Taken together, these data indicated that the contorsbody molecules P1AE0085, P1AE0086 and P1AE0087 have a similar potency than that of the control molecules, despite a slightly reduced binding capacity to OX40 and huFAP.

All in all, despite the different format, Contorsbodies (and especially CD134-0093) seem to have comparable, if not better properties regarding binding and T-cell activation, than the FAP targeted 2+1 anti OX40 antibodies described in Example 2.18.

Example 6

Preparation of bispecific antibodies with two antigen binding domains binding to 4-1BB and one antigen binding domain binding to FAP (FAP-4-1BB contorsbodies)

The generation and preparation of the FAP binders is described in WO 2012/020006 A2, which is incorporated herein by reference. For the 4-1BB binder, the VH and VL sequences of clone 20H4.9 were obtained in accordance with US 7,288,638 B2 or US 7,659,384 B2.

6.1 Preparation of FAP (4B9)-4-1BB (20H4.9) contorsbody P1AE1899

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in Figure 1A:

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- first fusion polypeptide (from N- to C-terminus): VH (4-1BB)-CH1, (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(4-1BB)-Ckappa, (GGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa

- 5 - second fusion polypeptide (from N- to C-terminus): VH (4-1BB)-CH1, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(4-1BB)-Ckappa, GGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The
10 knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 26 shows the amino acid sequences of the bispecific antibody P1AE1899.

Table 26: Sequences of P1AE1899

SEQ ID NO:	Description	Sequence
143	first fusion polypeptide (Fc knob)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQ SPEKGLEWIGEINHGGYVTYNPSLESRVTISVDTSKNQFSLK LSSVTAADTAVYYCARDYGPNGYDWYFDLWGRGTLTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALGAPIEKTKAKKGQPREP QVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSGFFLYSKLTVDKSRWQQGNVFCSS VMHEALHNHYTQKSLSLSPGKGGGGSGGGGSEIVLTQSPA TSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD ASNRATGIPARFSGSGGTDFLTISSELPEDFAVYYCQQRS NWPPALTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSGGGSGGGSGGGGSEVQLLESGLVQPGGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGAS TYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYC AKGWFGGFNYWGQGTLVTVSSASVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC
144	second fusion polypeptide (Fc hole)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQ SPEKGLEWIGEINHGGYVTYNPSLESRVTISVDTSKNQFSLK LSSVTAADTAVYYCARDYGPNGYDWYFDLWGRGTLTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT

		SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREP QVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSS VMHEALHNHYTQKSLSLSPGKGGGGSGGGGSEIVLTQSPA TLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD ASNRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCQQRS NWPPALTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSGGGSGGGSGGGGSEIVLTQSPGTLSPGER ATLSRASQSVTSSYLAWYQQKPGQAPRLINVGSRRTGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQ GTKVEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSLGTQTYICNVNHKPSNTKVDKKVEPKSC
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A schematic scheme of the assembled structure is shown in **Figure 1E** (OX40 to be replaced by 4-1BB).

6.2 Preparation of FAP (4B9)-4-1BB (20H4.9) contorsbody P1AE2051

- 5 A bispecific antibody comprising two fusion polypeptides was cloned as follows:
- first fusion polypeptide (from N- to C-terminus): VH(4-1BB)-CH1_EE (K147E, K213E), (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(4-1BB)-Ckappa_RK (E123R, Q124K), GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.
 - second fusion polypeptide (from N- to C-terminus): VH(4-1BB)-CH1_EE (K147E,
- 10 K213E), (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(4-1BB)-Ckappa_RK (E123R, Q124K), GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

- The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The
- 15 knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)). Furthermore, in the CH and Ckappa fused to the VL and VH of OX40, respectively, amino acid mutations (so-called charged residues) were introduced to prevent the generation of Bence Jones
- 20 proteins and to further facilitate the correct pairing, i.e negative charges in the CH1 domain (K147E, K213E, numbering according Kabat EU index) and positive charges in the CL domain of the anti-OX40 binder 49B4 (E123R and Q124K, numbering according to Kabat EU index).

Table 27 shows the amino acid sequences of the bispecific antibody P1AE2051.

Table 27: Sequences of P1AE2051:

SEQ ID NO:	Description	Sequence
145	first fusion polypeptide (Fc knob)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQ SPEKGLEWIGEINHGGYVTYNPSLESRVTISVDTSKNQFSLK LSSVTAADTAVYYCARDYGPNGYDWYFDLWGRGTLTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDEKVEPKSCGGGGSGGGGSDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQV YTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGKGGGGSGGGGSEIVLTQSPATLS LSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDAS NRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCQQRSN WPPALTFGGGTKEIKRTVAAPSVFIFPPSDRKLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSGGGSGGGSGGGGSEVQLLESGLVQPGGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGAS TYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC AKGWFGGFNYWGQGTILVTVSSASVAAPSVFIFPPSDEQLK SGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC
146	second fusion polypeptide (Fc hole)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQ SPEKGLEWIGEINHGGYVTYNPSLESRVTISVDTSKNQFSLK LSSVTAADTAVYYCARDYGPNGYDWYFDLWGRGTLTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDEKVEPKSCGGGGSGGGGSDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQV CTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGKGGGGSGGGGSEIVLTQSPATLSL SPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASN RATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCQQRSNWP PALTFGGGTKEIKRTVAAPSVFIFPPSDRKLKSGTASVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGGSGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLS CRASQSVTSSYLAWYQQKPGQAPRLINVGSRRTATGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTK VEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC

A schematic scheme of the assembled structure is shown in **Figure 1L** (OX40 to be replaced by 4-1BB).

6.3 Biochemical analysis of the molecules after purification

5 Table 28 summarizes the yield and final monomer content of the FAP-4-1BB contorsbodies.

Table 28 Biochemical analysis of the FAP 4-1BB contorsbodies

Construct	Monomer [%] (SEC)	Yield after ProtA [mg/l]
contorsbody P1AE1899	100	0.72
contorsbody P1AE2051	100	0.06

Example 7

10 Characterization of FAP-4-BB contorsbodies

7.1 Binding on human FAP (kinetic affinity)

Binding of bispecific FAP-4-1BB antibodies to human FAP was investigated by surface plasmon resonance using a BIACORE T100 instrument (GE Healthcare) as described in Example 3.2. For calculation of K_D and kinetic parameters the Langmuir 1:1 model was used.

15 **Table 29:** Binding of anti-FAP/anti-4-1BB antibodies to recombinant human FAP

Molecule	k_a (1/Ms)	k_d (1/s)	K_D (M)	R_{max} (RU)	$t_{1/2}$ (s)
Contorsbody P1AE1899	5.41E+04	2.80E-04	5.17E-09	18.8	2475.7
Contorsbody P1AE2051	4.40E+04	3.67E-04	8.32E-09	6.7	1891.1

Both molecules have similar K_D values.

7.2 NF- κ B activation in human 4-1BB and NF κ B-luciferase reporter gene expressing reporter cell line Jurkat-hu4-1BB-NF κ B-luc2

Agonistic binding of the 4-1BB (CD137) receptor to its ligand (4-1BBL) induces 4-1BB-downstream signaling via activation of nuclear factor kappa B (NF κ B) and promotes survival and activity of CD8 T cells (Lee HW, Park SJ, Choi BK, Kim HH, Nam KO, Kwon BS. 4-1BB promotes the survival of CD8 (+) T lymphocytes by increasing expression of Bcl-x(L) and Bfl-1. J Immunol 2002; 169:4882-4888). To monitor this NF κ B-activation mediated by 2+1 H2H anti-4-1BB, anti-FAP huIgG1 PGLALA bispecific antibody, Jurkat-hu4-1BB-NF κ B-luc2 reporter cell line was purchased from Promega (Germany). The cells were cultured as suspension cells in RPMI 1640 medium (GIBCO by Life Technologies, Cat No 42401-042) supplied with 10% (v/v) fetal bovine serum (FBS, GIBCO by Life Technologies, Cat.-No. 16000-044, Lot 941273, gamma irradiated mycoplasma free, heat inactivated), 2 mM L-alanyl-L-glutamine dipeptide (GlutaMAX-I, GIBCO by Life Technologies, Cat.-No. 35050-038), 1 mM Sodium Pyruvate (SIGMA-Aldrich Cat.-No. S8636), 1% (v/v) MEM-Non essential Aminoacid Solution 100x (SIGMA-Aldrich, Cat.-No. M7145), 600 μ g/ml G-418 (Roche, Cat.-No. 04727894001), 400 μ g/ml Hygromycin B (Roche, Cat.-No.: 10843555001) and 25 mM HEPES (Sigma Life Science, Cat.-No.: H0887-100 mL. For the assay, cells were harvested and resuspended in assay medium RPMI 1640 medium supplied with 10 % (v/v) FBS and 1 % (v/v) GlutaMAX-I. 10 μ L containing 2×10^3 Jurkat-hu4-1BB-NF κ B-luc2 reporter cells were transferred to each well of a sterile white 384-well flat bottom tissue culture plate with lid (Corning, Cat.-No.:3826). 10 μ L of assay medium containing titrated concentrations of the contorsbodies, 2+1 bispecific agonistic anti-4-1BB (20H4.9) x anti-FAP (4B9) huIgG1 P329GLALA antibody, anti-4-1BB (20H4.9) huIgG1 P329GLALA antibody, anti-4-1BB (20H4.9) huIgG4 and isotype control (DP47 hu IgG1 P329GLALA antibody) were added. Finally, 10 μ L of assay medium alone or containing 1×10^4 cells FAP-expressing cells, NIH/3T3-huFAP clone 19 (as described above) was supplied and plates were incubated for 6 hours at 37 °C and 5 % CO₂ in a cell incubator. 6 μ L freshly thawed One-Glo Luciferase assay detection solution (Promega, Cat.-No.: E6110) were added to each well and Luminescence light emission were measured immediately using Tecan microplate reader (500 ms integration time, no filter collecting all wavelength).

As shown in **Figure 23A**, in the absence of FAP expressing cells none of the molecules was able to induce strong human 4-1BB receptor activation in the Jurkat-hu4-1BB-NF κ B-luc2 reporter cell line, leading to NF κ B-activation and therefore Luciferase expression. In the presence of FAP-expressing cells like NIH/3T3-huFAP clone 19 (human-FAP-transgenic mouse fibroblast cell line) (see **Figure 23B**) crosslinking of bispecific 2+1 FAP 4-1BB contorsbodies as well as 2+1 bispecific agonistic anti-4-1BB (20H4.9) x anti-FAP (4B9) huIgG1 P329GLALA antibody (black filled star) led to a strong increase of NF κ B-activated Luciferase activity in the Jurkat-hu4-1BB-NF κ B-luc2 reporter cell line, which was above the activation mediated by the

untargeted control 4-1BB antibodies. EC₅₀ values and area under the curve (AUC) of activation curves are listed in **Table 30** and **Table 31**.

Table 30: EC₅₀ values of activation curves shown in Figure 23B

EC ₅₀ [nM]	NIH/3T3-huFAP clone 19
Contorsbody P1AE1899	0.070
Contorsbody P1AE2052	0.055
2+1 bispecific agonistic anti-4-1BB (20H4.9) x anti-FAP (4B9) huIgG1 P329GLALA antibody	0.020
4-1BB (20H4.9) huIgG1 P329GLALA	0.321
4-1BB (20H4.9) huIgG4	0.701
Untargeted (DP47) huIgG1 P329G LALA	n.d.

5 **Table 31: Values of area under the curve (AUC) of activation curves shown in Figure 23B**

AUC	NIH/3T3-huFAP clone 19
Contorsbody P1AE1899	36079
Contorsbody P1AE2052	36239
2+1 bispecific agonistic anti-4-1BB (20H4.9) x anti-FAP (4B9) huIgG1 P329GLALA antibody	53529
4-1BB (20H4.9) huIgG1 P329GLALA	15458
4-1BB (20H4.9) huIgG4	13032
Untargeted (DP47) huIgG1 P329G LALA	n.d.

Example 8

Preparation of bispecific antibodies with two antigen binding domains binding to CD40 and one antigen binding domain binding to FAP (FAP-CD40 contorsbodies)

10 The generation and preparation of the FAP binders is described in WO 2012/020006 A2, which is incorporated herein by reference. For the CD40 binder, the VH and VL sequences of clone 20H4.9 were obtained in accordance with SEQ ID NO:10 and SEQ ID NO:16 of WO 2006/128103.

8.1 Preparation of FAP (4B9)-CD40 contorsbody P1AE1799

A bispecific antibody comprising two fusion polypeptides was cloned as depicted in

Figure 1A:

- first fusion polypeptide (from N- to C-terminus): VH (CD40)-CH1, (G4S)2 connector ,
- 5 IgG1 hinge, Fc knob, (G4S)2 connector, VL(CD40)-Ckappa, (GGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa
- second fusion polypeptide (from N- to C-terminus): VH (CD40)-CH1, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(CD40)-Ckappa, GGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.
- 10 The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V
- 15 mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 32 shows the amino acid sequences of the bispecific antibody P1AE1799.

Table 32: Sequences of P1AE1799

SEQ ID NO:	Description	Sequence
155	first fusion polypeptide (Fc knob)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQA PGKGLEWVGRVIPNAGGTSYNQKFKGRFTISVDNSKNTAY LQMNSLRAEDTAVYYCAREGIYWWGQGTITVTSSASTKG PSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTISWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCGGGSGGGGSDKTHCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPC RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHN HYTQKSLSLSPGKGGGSGGGGSDIQMTQSPSSLSASVGD RVTITCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSN RFGSGVPSRFGSGSGTDFTLTISLQPEDFATYFCSQTTHVP WTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLS SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSGGGSGGGSGGGSEVQLLESGGGLVQPGGSLRLSC AASGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYYAD SVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKGF GGFNYWGQGTITVTSSASVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR

		GEC
156	second fusion polypeptide (Fc hole)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQA PGKGLEWVGRVIPNAGGTSYNQKFKGRFTISVDNSKNTAY LQMNSLRAEDTAVYYCAREGIYWWGQGTITVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPSS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSSLSASVGD RVTITCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSN RFGSVPSRFSGSGSGTDFLTISLQPEDFATYFCSQTTHVP WTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSC RASQSVTSSYLAWYQQKPGQAPRLINVGSRRTATGIPDRFS GSGSGTDFLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKV EIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSC

A schematic scheme of the assembled structure is shown in **Figure 1E** (OX40 to be replaced by CD40).

8.2 Preparation of FAP (4B9)-CD40 contorsbody P1AE1902

- 5 A bispecific antibody comprising two fusion polypeptides was cloned as follows:
- first fusion polypeptide (from N- to C-terminus): VH (CD40)-CH1, (G4S)2 connector , IgG1 hinge, Fc knob, (G4S)2 connector, VL(CD40)-Ckappa, (GGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1
 - second fusion polypeptide (from N- to C-terminus): VH (CD40)-CH1, (G4S)2 connector,
 - 10 IgG1 hinge, Fc hole, (G4S)2 connector, VL(CD40)-Ckappa, GGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.

- The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The
- 15 knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 33 shows the amino acid sequences of the bispecific antibody P1AE1902.

Table 33: Sequences of P1AE1902:

SEQ ID NO:	Description	Sequence
157	first fusion polypeptide (Fc knob)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQA PGKGLEWVGRVIPNAGGTSYNQKFGRFTISVDNSKNTAY LQMNSLRAEDTAVYYCAREGIYWWGQGTITVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPC RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSSLSASVGD RVTITCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSN RFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCSQTTHVP WTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSC RASQSVTSSYLAWYQQKPGQAPRLLINVGSRRTATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKV EIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSC
158	second fusion polypeptide (Fc hole)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQA PGKGLEWVGRVIPNAGGTSYNQKFGRFTISVDNSKNTAY LQMNSLRAEDTAVYYCAREGIYWWGQGTITVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPPS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSCVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSSLSASVGD RVTITCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSN RFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCSQTTHVP WTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSGGGSGGGSGGGGSEVQLLESGGGLVQPGGSLRLSC AASGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYYAD SVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKGWF GGFNYWGQGLVTVSSASVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC

8.3 Preparation of FAP (4B9)-CD40 contorsbody P1AE1800

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- first fusion polypeptide (from N- to C-terminus): VH (CD40)-CH1, (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(CD40)-Ckappa, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.
- second fusion polypeptide (from N- to C-terminus): VL(CD40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VH(CD40)-CH1, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 34 shows the amino acid sequences of the bispecific antibody P1AE1800.

Table 34: Sequences of P1AE1800

SEQ ID NO:	Description	Sequence
159	first fusion polypeptide (Fc knob)	EVQLLES GGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQA PGKGLEWVGRVIPNAGGTSYNQKFKGRFTISVDNSKNTAY LQMNSLRAEDTAVYYCAREGIYWWGQGTTVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYCKKVS NKALGAPIEKTISKAKGQPREPQVYTLPPC RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHN HYTQKSLSLSPGKGGGGSGGGGSDIQMTQSPSSLSASVGD RVITTCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSN RFSGVPSRFSGSGGTDFLTISLQPEDFATYFCSQTTHVP WTFGGGTIKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSGGGGSGGGSGGGGSEVQLLES GGGLVQPGGSLRLSC AASGFTFSYAMSWVRQAPGKGLEWVSAIIGSGASTYYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGWF GGFNYWGQGT LVTVSSASVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC

160	second fusion polypeptide (Fc hole)	DIQMTQSPSSLSASVGDRVITTCRSSQSLVHSNGNTFLHWY QQKPGKAPKLLIYTVSNRFGVPSRFSGSGSGTDFLTISL QPEDFATYFCSQTTHVPWTFGGGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPPS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLVSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSEVQLLESGGGLVQPGGS LRLSCAASGYSFTGYIHWVRQAPGKGLEWVGRVIPNAG GTSYNQKFKGRFTISVDNSKNTAYLQMNSLRAEDTAVYY CAREGIYWWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC GGGGSGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLS CRASQSVTSSYLAWYQQKPGQAPRLINVGSRRTATGIPDRF SGSGSGTDFLTISRLEPEDFAVYYCQQGIMLPPTFGQGTK VEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC
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8.4 Preparation of FAP (4B9)-CD40 contorsbody P1AE2052

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- first fusion polypeptide (from N- to C-terminus): VL(CD40)-Ckappa, (G4S)2 connector,
- 5 IgG1 hinge, Fc knob, (G4S)2 connector, VH(CD40)-CH1, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.
- second fusion polypeptide (from N- to C-terminus): VL(CD40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VH(CD40)-CH1, GGGGSGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

- 10 The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V
- 15 mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 35 shows the amino acid sequences of the bispecific antibody P1AE2052.

Table 35: Sequences of P1AE2052

SEQ ID	Description	Sequence
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NO:		
161	first fusion polypeptide (Fc knob)	DIQMTQSPSSLSASVGDRVTTICRSSQSLVHSNGNTFLHWY QQKPGKAPKLLIYTVSNRFGVPSRFSGSGSGTDFTLTISL QPEDFATYFCSQTTHVPWTFGGGKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPC RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSEVQLLESGGGLVQPGGS LRLSCAASGYSFTGYIHWVRQAPGKGLEWVGRVIPNAG GTSYNQKFKGRFTISVDNSKNTAYLQMNSLRAEDTAVYY CAREGIYWWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC GGGGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRL SCAASGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYY ADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKG WFGGFNYWGQGLTVTVSSASVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
162	second fusion polypeptide (Fc hole)	DIQMTQSPSSLSASVGDRVTTICRSSQSLVHSNGNTFLHWY QQKPGKAPKLLIYTVSNRFGVPSRFSGSGSGTDFTLTISL QPEDFATYFCSQTTHVPWTFGGGKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPPS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSEVQLLESGGGLVQPGGS LRLSCAASGYSFTGYIHWVRQAPGKGLEWVGRVIPNAG GTSYNQKFKGRFTISVDNSKNTAYLQMNSLRAEDTAVYY CAREGIYWWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC GGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLS CRASQSVTSSYLAWYQQKPGQAPRLLINVGSRRTATGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTK VEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC

8.5 Preparation of FAP (4B9)-CD40 contorsbody P1AE1901

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- first fusion polypeptide (from N- to C-terminus): VL(CD40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VH(CD40)-CH1, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.
- second fusion polypeptide (from N- to C-terminus): VL(CD40)-Ckappa, (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VH(CD40)-CH1, GGGGSGGGGSGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.

The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)).

Table 36 shows the amino acid sequences of the bispecific antibody P1AE1901.

Table 36: Sequences of P1AE1901

SEQ ID NO:	Description	Sequence
163	first fusion polypeptide (Fc knob)	DIQMTQSPSSLSASVGDRTTITCRSSQSLVHSNGNTFLHWY QQKPGKAPKLLIYTVSNRFSGVPSRFSGSGGTDFLTISL QPEDFATYFCSQTTHVPWTFGGGTKVEIKRTVAAPSVFI PSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPC RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHN HYTQKSLSLSPGKGGGGSGGGGSEVQLLESGGGLVQPGGS LRLSCAASGYSFTGYIHWVRQAPGKLEWVGRVIPNAG GTSYNQKFKGRFTISVDNSKNTAYLQMNSLRAEDTAVYY CAREGIYWWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC GGGGSGGGGSGGGSGGGGSEIVLTQSPGTLISLSPGERATLS CRASQSVTSSYLAWYQQKPGQAPRLLINVGSRRTATGIPDRF SGSGSGTDFLTISRLEPEDFAVYYCQQGIMLPPTFGQGTK VEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC

164	second fusion polypeptide (Fc hole)	DIQMTQSPSSLSASVGDRVTTICRSSQSLVHNSGNTFLHWY QKPGKAPKLLIYTVSNRFGVPSRFGSGSGTDFLTISL QPEDFATYFCSQTTHVPWTFGGGKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGECGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPSS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLVSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSEVQLLESGGGLVQPGGS LRLSCAASGYSFTGYIHWVRQAPGKGLEWVGRVIPNAG GTSYNQKFKGRFTISVDNSKNTAYLQMNSLRAEDTAVYY CAREGIYWWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC GGGGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRL SCAASGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYY ADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKG WFGGFNYWGQGLTVTVSSASVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
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8.6 Preparation of FAP (4B9)-CD40 contorsbody P1AE2255

A bispecific antibody comprising two fusion polypeptides was cloned as follows:

- first fusion polypeptide (from N- to C-terminus): VH(CD40)-CH1_EE (K147E, K213E),
 5 (G4S)2 connector, IgG1 hinge, Fc knob, (G4S)2 connector, VL(CD40)-Ckappa_RK (E123R, Q124K), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:84) connector, VH(FAP)-Ckappa.
- second fusion polypeptide (from N- to C-terminus): VH(CD40)-CH1_EE (K147E, K213E), (G4S)2 connector, IgG1 hinge, Fc hole, (G4S)2 connector, VL(CD40)-Ckappa_RK (E123R, Q124K), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:84) connector, VL(FAP)-CH1.

- 10 The Pro329Gly, Leu234Ala and Leu235Ala mutations have been introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in International Patent Appl. Publ. No. WO 2012/130831. The knobs into hole heterodimerization technology was used with the S354C/T366W mutations in the CH3 domain of the knob chain and the corresponding Y349C/T366S/L368A/Y407V
- 15 mutations in the CH3 domain of the hole chain (Carter, J Immunol Methods 248, 7-15 (2001)). Furthermore, in the CH and Ckappa fused to the VL and VH of OX40, respectively, amino acid mutations (so-called charged residues) were introduced to prevent the generation of Bence Jones proteins and to further facilitate the correct pairing, i.e negative charges in the CH1 domain

(K147E, K213E, numbering according Kabat EU index) and positive charges in the CL domain of the anti-OX40 binder 49B4 (E123R and Q124K, numbering according to Kabat EU index).

Table 37 shows the amino acid sequences of the bispecific antibody P1AE2255.

Table 37: Sequences of P1AE2255:

SEQ ID NO:	Description	Sequence
165	first fusion polypeptide (Fc knob)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQA PGKGLEWVGRVIPNAGGTSYNQKFKGRFTISVDNSKNTAY LQMNSLRAEDTAVYYCAREGIYWWGQGTITVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDEKVEPKSCGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPC RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSDIQTQSPSSLSASVGD RVTITCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSN RFSGVPSRFSGSGSGTDFLTITSSLPEDFATYFCSQTTHVP WTFGGGTKVEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSC AASGFTFSYAMSWVRQAPGKGLEWVSAIIGSGASTYYAD SVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKWF GGFNYWGQGTITVTVSSASVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
166	second fusion polypeptide (Fc hole)	EVQLLESGGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQA PGKGLEWVGRVIPNAGGTSYNQKFKGRFTISVDNSKNTAY LQMNSLRAEDTAVYYCAREGIYWWGQGTITVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDEKVEPKSCGGGGSGGGGSDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPPS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGKGGGGSGGGGSDIQTQSPSSLSASVGD RVTITCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSN RFSGVPSRFSGSGSGTDFLTITSSLPEDFATYFCSQTTHVP WTFGGGTKVEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSC RASQSVTSSYLAWYQQKPGQAPRLLINVGSRRTATGIPDRFS

-167-

		GSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKV EIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSC
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A schematic scheme of the assembled structure is shown in **Figure 1L** (OX40 to be replaced by CD40).

8.7 Biochemical analysis of the molecules after purification

5 Table 38 summarizes the yield and final monomer content of the FAP-4-1BB contorsbodies.

Table 38 Biochemical analysis of the FAP 4-1BB contorsbodies

Construct	Monomer [%] (SEC)	Titer [mg/l]
contorsbody P1AE1799	100	3.40
contorsbody P1AE1902	100	4.42
contorsbody P1AE1800	98.8	2.44
contorsbody P1AE2052	100	4.06
contorsbody P1AE1901	100	3.60
contorsbody P1AE2255	100	1.82

Example 9

10 Characterization of FAP CD40 antibodies

9.1 Binding to human CD40

The capacity of the bispecific constructs to bind human CD40 was assessed by surface plasmon resonance (SPR). All SPR experiments were performed on a Biacore T200 (Biacore) at 25 °C with HBS-EP as running buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 15 0.005% Surfactant P20, (Biacore). Association was measured by injection of human CD40 extra cellular domain in various concentrations in solution for 300 sec at a flow of 30 µl/min starting with 300 nM in 1:3 dilutions. The dissociation phase was monitored for up to 1200 sec and triggered by switching from the sample solution to running buffer. The surface was regenerated

by 60 sec washing with a Glycine pH 2.1 solution at a flow rate of 30 μ l/min. Bulk refractive index differences were corrected by subtracting the response obtained from a goat anti human F(ab')₂ surface. Blank injections are also subtracted (= double referencing). For calculation of apparent K_D and other kinetic parameters the Langmuir 1:1 model was used. The apparent K_d was calculated using the Biacore™ B4000 evaluation software (version 1.1).

Table 39: Binding of bispecific CD40 x FAP Contorsbodies to recombinant human CD40_ECD (Biacore)

Molecule	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	t 1/2 (s)
contorsbody P1AE1800	1.35E+06	1.27E-02	9.43E-09	5.2	55
contorsbody P1AE1799	9.57E+05	1.51E-02	1.57E-08	10.7	46
contorsbody P1AE1901	9.91E+05	6.92E-03	6.98E-09	12.3	100
contorsbody P1AE1902	7.76E+05	1.45E-02	1.87E-08	9.9	48
contorsbody P1AE2052	9.45E+05	6.81E-03	7.20E-09	10.9	102
contorsbody P1AE2255	9.18E+05	1.59E-02	1.73E-08	14.2	44

9.2 Binding on human FAP (kinetic affinity)

Binding of bispecific FAP-OX40 antibodies to human FAP was investigated by surface plasmon resonance using a BIACORE T100 instrument (GE Healthcare). Around 12000 resonance units (RU) of the capturing system (15 μ g/ml anti- histidine antibody; Order Code: 28995056; GE Healthcare Bio-Sciences AB, Sweden) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 4.5 by using an amine coupling kit supplied by the GE Healthcare. Running buffer for Immobilization was HBS-N pH 7.4 (10 mM HEPES, 150 mM NaCl, pH 7.4, GE Healthcare). For the following kinetic characterization running buffer was PBS-P pH 7.4 (20 mM phosphate buffer, 2.7 mM KCl, 137 mM NaCl, 0.05% surfactant P20). The flow cell was set to 25 °C - and the sample block set to 12 °C - and primed with running buffer twice. The recombinant human FAP was captured by injecting a 25 μ g/ml solution for 60 s at a flow rate of 5 μ l/min. Association was measured by injection of the bispecific antibody for 120 s at a flow rate of 30 μ l/min starting with 300 nM in 1:2 dilution. The dissociation phase was monitored for up to 720 s and triggered by switching from the sample solution to running buffer. The surface was regenerated by washing with 10 mM Glycine pH 1.5 for 60 s at a flow rate of 30 μ l/min. Bulk refractive index differences were corrected by subtracting the response obtained from an anti-histidine surface. Blank injections are also subtracted (= double referencing). For calculation of K_D and kinetic parameters the Langmuir 1:1 model was used.

Table 40: Binding of anti-FAP/anti-CD40 antibodies to recombinant human FAP

Molecule	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	t 1/2 (sec)
contorsbody P1AE1800	8.57E+04	7.78E-04	9.07E-09	18.4	891
contorsbody P1AE1799	5.53E+04	2.53E-04	4.58E-09	28.9	2741
contorsbody P1AE1901	3.82E+04	2.69E-04	7.05E-09	32.0	2578
contorsbody P1AE1902	5.41E+04	2.88E-04	5.33E-09	26.0	2405
contorsbody P1AE2052	3.56E+04	2.96E-04	8.31E-09	28.2	2345
contorsbody P1AE2255	5.28E+04	2.35E-04	4.44E-09	36.7	2955

9.3 Simultaneous Binding on human CD40 and human FAP (kinetic affinity)

The capacity of binding simultaneously human CD40 and human FAP was also assessed by surface plasmon resonance (SPR) using a BIACORE T100 instrument (GE Healthcare). Around 8000 resonance units (RU) of the capturing system (20 µg/ml anti-human IgG (Fc); Order Code: BR100839; GE Healthcare Bio-Sciences AB, Sweden) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. Running buffer was PBS-P pH 7.4 (20 mM phosphate buffer, 2.7 mM KCl, 137 mM NaCl, 0.05% Surfactant P20). The flow cell was set to 25 °C - and the sample block set to 12 °C - and primed with running buffer twice. The bispecific antibody was captured by injecting a 2 µg/ml solution for 60 seconds at a flow rate of 5 µl/min. Association was measured by injection of the first analyte (human CD40 or human FAP, respectively) for 120 seconds at a flow rate of 30 µl/min. Then the second analyte (human FAP or human CD40, respectively) was injected with a flow rate of 30 µl/min for 120 seconds. The dissociation phase was monitored for up to 720 seconds and triggered by switching from the sample solution to running buffer. The surface was regenerated by washing with 3 M MgCl₂ for 60 seconds at a flow rate of 10 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from an anti-human IgG (Fc) surface. Blank injections are also subtracted (= double referencing). For calculation of K_D and kinetic parameters the Langmuir 1:1 model was used. All FAP-CD40 contorsbodies were able to bind simultaneously and independently to both antigens.

Example 10

Functional properties of FAP-targeted anti-human CD40 binding molecules

10.1 Activation of human B cells by FAP-targeted anti-human CD40 binding molecules using FAP-coated Dynabeads® as source of antigen

- 5 B cells were isolated from buffy coats obtained from the Stiftung Zürcher Blutspendedienst SRK. In order to isolate peripheral blood mononuclear cells (PBMCs), a buffy coat of 50 mL was diluted in the same volume of PBS (gibco, Cat. No. 10010023). 50 mL polypropylene centrifuge tubes (TPP, Cat. No. 91050) were supplied with 15 mL of Lymphoprep™ (STEMCELL Technologies, Cat. No. 07851) and 25 mL of the buffy coat solution per tube were
- 10 carefully layered above the Lymphoprep™. The tubes were centrifuged at 2000 rpm for 24 minutes at room temperature with low acceleration and without break. Afterwards, the PBMCs were collected from the interface, washed three times with PBS, resuspended in 10 mL of PBS and cells were analyzed for cell type and number with a Beckman Coulter cell counter Ac·T™ 5diff OV (Beckman Coulter, Cat. No. 6605580). Prior to the B cell isolation from the PBMCs,
- 15 the CD14 positive fraction was removed by magnetic labeling of the CD14 positive cells with CD14 microbeads (Miltenyi, Cat. No. 130-050-201) and subsequent isolation with the autoMACS® Pro Separator (Miltenyi, Cat. No. 130-092-545). The CD14 negative fraction was used for subsequent B cell isolation with the Miltenyi B cell isolation kit II (Cat. No. 130-091-151) and autoMACS® separation. 1×10^5 B cells in 100 μ l of R10 medium consisting of Roswell
- 20 Park Memorial Institute (RPMI) medium 1640 (gibco, Cat. No. 31870-025) supplied with 10 % (v/v) Fetal Bovine Serum (FBS) (life technologies, Cat. No. 16140, Lot No. 1797306A), 1% (v/v) Penicillin/Streptomycin (gibco, Cat. No. 15070-063), 1% (v/v) L-Glutamine (gibco, Cat. No. 25030-024), 1 % (v/v) Sodium-Pyruvate (gibco, Cat. No. 11360-039), 1 % (v/v) MEM non-essential amino acids (gibco, Cat. No. 11140-035) and 50 μ M β -Mercaptoethanol (gibco, Cat.
- 25 No. 31350-010) were added per well of a 96-well flat-bottom plate. Streptavidin Dynabeads® (ThermoFisher Scientific, Cat. No.: 11205D) were coated with biotinylated human FAP (produced in-house, binding capacity of 6.5×10^4 beads: 0.01 μ g of protein) according to the manufacturer's protocol and added to the B cells in a bead to cell ratio of 2:1 in 50 μ l of R10 medium. As control non-coated beads were added to the B cells.
- 30 FAP-targeted anti-human CD40 antibodies were added in 50 μ l of R10 medium to the B cells at concentrations ranging from 6.7 to 0.003 nM (3x dilution series). As a positive FAP-independent control the agonistic anti-human CD40 antibody SGN40 (IgG1, INN: Dacetuzumab) was used. Since it is described in the literature that the SGN40 antibody requires Fc receptor cross-linking for biological activity (C. Law et al., Cancer Res 2005, 65, 8331-8338), the
- 35 antibody was incubated with a cross-linking goat anti-human IgG Fc γ fragment specific F(ab')₂

fragment (Jackson ImmunoResearch, Cat. No. 109-006-008) for 30 minutes before addition to the B cells. After 48 hours, cells were transferred into a 96-well round-bottom plate, washed once with PBS and incubated with 50 µl of 3 µg/mL of Fc receptor blocking Mouse IgG Isotype Control (ThermoFisher Scientific, Cat. No.10400C) in PBS. After 15 minutes of incubation at 4°C, cells were washed with PBS and 50 µl of a mixture of fluorescently labelled antibodies in PBS was added to the cells. The following fluorescently labelled antibodies were used: anti-human CD80 BV605 (BD Biosciences, clone L307.4, Cat. No. 563315), anti-human CD69 Alexa Fluor® 488 (Biolegend, clone FN50, Cat. No. 310916), anti-human CD14 PerCP-Cy5.5 (Biolegend, clone HCD14, Cat. No. 325622), anti-human CD3 PerCP-Cy5.5 (Biolegend, clone UCHT1, Cat. No. 300430), anti-human CD86 PE-CF594 (BD Biosciences, clone FUN-1, Cat. No. 562390), anti-HLA-DR BUV395 (BD Biosciences, clone G46-6, Cat. No. 564040) and anti-human CD19 APC-H7 (BD Biosciences, clone SJ25C1, Cat. No. 560177). In order to distinguish between live and dead cells, the viability dye Zombie Aqua™ (Biolegend, Cat. No. 423102) was added to the antibody mixture. After 30 minutes of incubation at 4°C, cells were washed twice with PBS and resuspended in 200 µl of PBS. Cells were analyzed the same day using a 5-laser LSR-Fortessa (BD Bioscience with DIVA software). Data analysis was performed using the FlowJo version 10 software (FlowJo LLC). Live (aqua negative) cells, negative for CD14 and CD3 and positive for CD19 were analyzed for CD69, CD80, CD86 and HLA-DR expression.

B cells analyzed after 2 days of incubation with agonistic anti-CD40 contorsbodies or cross-linked SGN40 antibody showed an increase in CD69, CD80, CD86 and HLA-DR expression for all tested constructs (see **Figures 24A to 24H**). Upregulation of these expression markers was dependent on FAP in case of the different FAP-targeted contorsbodies and increase of expression induced by these FAP-dependent antibodies was comparable or slightly lower to the increase induced by the cross-linked SGN40 antibody.

Claims

1. A bispecific antibody consisting of two fusion polypeptides and comprising two antigen
binding domains capable of specific binding to a first target and one antigen binding domain
5 capable of specific binding to a second target comprising
 - (a) a first fusion polypeptide comprising a first part of a first antigen binding domain
capable of specific binding to the first target, a spacer domain, a second part of a first
antigen binding domain capable of specific binding to the first target and a first part
of an antigen binding domain capable of specific binding to the second target,
10 wherein
 - the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
 - the first part of the first antigen binding domain capable of specific binding to the
first target is fused either directly or via a first peptide linker to the N-terminus of the
spacer domain,
 - 15 - the second part of the first antigen binding domain capable of specific binding to
the first target is fused either directly or via a second peptide linker to the C-terminus
of the spacer domain, and
 - the first part of an antigen binding domain capable of specific binding to a second
target is fused either directly or via a third peptide linker to the C-terminus of the
20 second part of the first antigen binding domain capable of specific binding to the first
target or is fused either directly or via a third peptide linker to the N-terminus of the
first part of the first antigen binding domain capable of specific binding to the first
target, and
 - (b) a second fusion polypeptide comprising a first part of a second antigen binding domain
25 capable of specific binding to a first target, a spacer domain, a second part of the
second antigen binding domain capable of specific binding to a first target and the
second part of an antigen binding domain capable of specific binding to a second
target, wherein
 - the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
 - 30 - the first part of the second antigen binding domain capable of specific binding to a
first target is fused either directly or via a first peptide linker to the N-terminus of the
spacer domain,

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- the second part of the second antigen binding domain capable of specific binding to a first target is fused either directly or via a second peptide linker to the C-terminus of the spacer domain, and
 - the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the second antigen binding domain capable of specific binding to a first target or is fused either directly or via a third peptide linker to the N-terminus of the first part of the second antigen binding domain capable of specific binding to a first target,
- and wherein the first part and the second part of the antigen binding domain capable of specific binding to the second target are associated with each other to form the antigen binding domain capable of specific binding to the second target and wherein the first part and the second part of the first and second antigen binding domain capable of specific binding to the first target are associated with each other to form a circular fusion polypeptide, and
- wherein the spacer domain of the first fusion polypeptide and the spacer domain of the second fusion polypeptide are associated covalently to each other by a disulfide bond and comprise modifications promoting the association of the first and second fusion polypeptide.
2. The bispecific antibody of claim 1, wherein in the first fusion polypeptide the first part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the first antigen binding domain capable of specific binding to the first target and wherein in the second fusion polypeptide the second part of an antigen binding domain capable of specific binding to a second target is fused either directly or via a third peptide linker to the C-terminus of the second part of the first antigen binding domain capable of specific binding to the first target.
 3. The bispecific antibody of claims 1 or 2, wherein the third peptide linker connecting the first part or the second part of an antigen binding domain capable of specific binding to a second target comprises at least 15 amino acids.
 4. The bispecific antibody of any one of claims 1 to 3, wherein the first fusion polypeptide comprises the heavy chain variable domain of the antigen binding domain capable of specific binding to a second target and the second fusion polypeptide comprises the antibody light chain variable domain of the antigen binding domain capable of specific binding to a second target or vice versa.

5. The bispecific antibody of any one of claims 1 to 4, wherein in both the first fusion polypeptide and the second fusion polypeptide the first part of the antigen binding domain capable of specific binding to the first target is an antibody heavy chain Fab fragment and the second part of the antigen binding domain capable of specific binding to the first target is an antibody light chain Fab fragment.
6. The bispecific antibody of any one of claims 1 to 5, wherein the spacer domain comprises an antibody hinge region or a fragment thereof, an antibody CH2 domain, and an antibody CH3 domain or a fragment thereof.
7. The bispecific antibody of any one of claims 1 to 6, wherein the spacer domain comprises an antibody hinge region or a fragment thereof and an IgG1 Fc domain.
8. The bispecific antibody of any one of claims 1 to 7, wherein the spacer domain of the first fusion polypeptide comprises holes and the spacer domain of the second fusion polypeptide comprises knobs according to the knobs into hole method.
9. The bispecific antibody of any one of claims 1 to 8, wherein the IgG1 Fc domain comprises amino acid substitutions L234A, L235A and P329G (numbering according to Kabat EU index).
10. The bispecific antibody of any one of claims 1 to 9, wherein the antigen binding domain capable of specific binding to a second target is an antigen binding domain capable of specific binding to a tumor associated antigen.
11. The bispecific antibody of any one of claims 1 to 10, wherein the antigen binding domain capable of specific binding to a second target is an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP).
12. The bispecific antibody of any one of claims 1 to 11, 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 acid sequence of SEQ ID NO:1, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:3, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:4, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:5, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:6, or
 - (b) a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:11, and a

light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:12, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:13, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:14.

13. The bispecific antibody of any one of claims 1 to 12, wherein the antigen binding domain capable of specific binding to FAP comprises
- 5 (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 NO:7, 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:8, or
- 10 (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 NO:15, 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:16.
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14. The bispecific antibody of any one of claims 1 to 13, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to OX40.
15. The bispecific antibody of any one of claims 1 to 14, wherein the antigen binding domain capable of specific binding to OX40 comprises
- 20 (a) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:35, or
- 25 (b) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:34, or
- 30 (c) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:23, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid
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- sequence of SEQ ID NO:28, (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:36, or
- (d) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:24, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (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:37, or
- (e) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (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:25, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or
- (f) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (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:26, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or
- (g) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (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:27, 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:33, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:39.
16. The bispecific antibody of any one of claims 1 to 15, wherein the antigen binding domain capable of specific binding to OX40 comprises
- (a) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:41, or
- (b) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:42 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:43, or
- (c) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:44 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:45, or

- (d) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:46 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:47, or
- 5 (e) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:48 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:49, or
- (f) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:50 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:51, or
- 10 (g) a heavy chain variable region (V_HOX40) comprising an amino acid sequence of SEQ ID NO:52 and a light chain variable region (V_LOX40) comprising an amino acid sequence of SEQ ID NO:53.
17. The bispecific antibody of any one of claims 1 to 15, wherein the antigen binding domain capable of specific binding to OX40 comprises
- 15 (a) a heavy chain variable region (V_HOX40) 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:40, and a light chain variable region (V_LOX40) 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:41.
- 20 18. The bispecific antibody of any one of claims 1 to 13, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to 4-1BB.
19. The bispecific antibody of any one of claims 1 to 13 or 18, wherein the antigen binding domain capable of specific binding to 4-1BB comprises a heavy chain variable region (V_H4-1BB) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, (ii)
- 25 CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:137, and a light chain variable region (V_L4-1BB) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and (vi)
- 30 CDR-L3 comprising the amino acid sequence of SEQ ID NO:140.
20. The bispecific antibody of any one of claims 1 to 13 or 18 or 19, wherein the antigen binding domain capable of specific binding to 4-1BB comprises a heavy chain variable region (V_H4-1BB) 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:141, and a light

chain variable region (V_L4-1BB) 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:142.

21. The bispecific antibody of any one of claims 1 to 13, wherein the antigen binding domain capable of specific binding to a first target is an antigen binding domain capable of specific binding to CD40.
22. The bispecific antibody of any one of claims 1 to 13 or 21, 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:147, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:148, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:149, and a light chain variable region (V_LCD40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:150, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:151, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:152.
23. The bispecific antibody of any one of claims 1 to 13 or 21 or 22, wherein the antigen binding domain capable of specific binding to CD40 comprises a heavy chain variable region (V_HCD40) 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:153, and a light chain variable region (V_LCD40) 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:154.
24. The bispecific antibody of any one of claims 1 to 13 or 21 or 22, wherein the antigen binding domain capable of specific binding to a first target is an 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:167, SEQ ID NO:168, SEQ ID NO:169 and SEQ ID NO:170, and a light chain variable region (V_LCD40) comprising the 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, or
- (ii) a heavy chain variable region (V_HCD40) 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:179 and SEQ ID NO:180, and a light chain variable region (V_LCD40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, and SEQ ID NO:184.

25. Isolated nucleic acid encoding the bispecific antibody of any one of claims 1 to 24.
26. A vector or host cell comprising the nucleic acid of claim 25.
27. A method of producing a bispecific antibody culturing the host cell of claim 26 under
5 conditions suitable for the expression of the bispecific antibody.
28. A pharmaceutical composition comprising the bispecific antibody of any one of claims 1 to 24 and a pharmaceutically acceptable excipient.
29. The bispecific antibody of any one of claims 1 to 24 or the pharmaceutical composition of claim 28 for use as medicament.
- 10 30. The bispecific antibody of any one of claims 1 to 24 or the pharmaceutical composition of claim 28 for use in treating cancer or infectious diseases.
31. Use of the bispecific antibody of any one of claims 1 to 24 or the pharmaceutical composition of claim 28 in the manufacture of a medicament for use
15 (i) in stimulating T cell response,
(ii) in supporting survival of activated T cells,
(iii) in the treatment of infections,
(iv) in the treatment of cancer,
(v) in delaying progression of cancer, or
(vi) in prolonging the survival of a patient suffering from cancer.
- 20 32. A method of treating an individual having cancer or infectious diseases comprising administering to the individual an effective amount of the bispecific antibody of any one of claims 1 to 24 or the pharmaceutical composition of claim 28.
33. The method of claim 32, further comprising administering an additional therapeutic agent to the individual.